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STUDIES ON THE RESISTANCE OF WHEAT STARCH TO DIASTATIC ACTION[1]

BY J. G. MALLOCH[2]

Abstract

The diastatic activity of wheat flour has been definitely resolved into two independent variable factors: (1) concentration and activity of the enzyme, (2) resistance of starch to hydrolysis. The method for the measurement of starch resistance involves the inactivation of the natural diastase with sodium tungstate, washing out the excess precipitant, and the addition of a fixed amount of taka-diastase. By the use of this method it has been found that continued grinding or extraction with ether decreases the resistance of the starch. Both diastatic activity and starch resistance are affected by the conditions under which the wheat is grown. The increase in diastatic activity when wheat is sprouted is due mainly to a change in the quantity or nature of the enzyme. Several modifications in Rumsey's method for diastatic activity are recommended.

Introduction

For the past few years extensive experiments on the effects of environment on the quality of wheat have been conducted by the Department of Field Crops of the University of Alberta. In these experiments the baking test has, of course, been the principal measure of quality. This has been supplemented by other determinations with a view to explaining the results of the baking test and for the purpose of studying the individual factors influencing these results. Among other things, determinations of the diastatic activity of the flour have been made.

There is ample evidence (20, 28, 54, 63) that diastatic activity may have a great influence on the quality of the bread obtained from any flour. It is true that the use of malt preparations in baking practice has somewhat lessened the importance of diastatic activity from a commercial point of view. However, a moderately high natural diastatic activity seems desirable and therefore the environmental factors which may affect the diastatic activity should be studied.

[1]*Manuscript received January 31, 1929.*
Contribution from the laboratories of the University of Alberta, Edmonton, Alberta, Canada, with financial assistance from the National Research Council of Canada. The present paper is being issued as Paper No. 3 of The Associate Committee on Grain Research and represents a thesis presented in partial fulfilment of the requirements for the degree of Ph.D. at the University of Minnesota.
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Rumsey's method (63) for the determination of diastatic activity was tentatively adopted for these investigations. It was found necessary, however, to make slight technical modifications and these will be dealt with in a later section of this paper. This method makes use of the determination of the sugar produced in a sample of flour by autolysis under controlled conditions. The results are therefore affected both by the character of the substrate and the nature of the enzyme, as well as by external factors such as the pH.

Until recently cereal chemists have been in the habit of looking on the starch as being essentially the same in all samples of flour. This has led rather commonly to the interpretation of the variation in the results of the Rumsey determination as variation in the activity of the enzyme. That this interpretation is entirely in error is revealed by a review of the literature.

The present investigation was undertaken in order to gather information on the variability of the resistance to diastatic action of the starch from different samples of wheat flour. A method was devised for the measurement of starch resistance which makes it possible to resolve diastatic activity, as determined by the modified Rumsey method, into its two main components, relating respectively to the enzyme and the substrate.

Review of Literature

It is only recently that any attempt has been made to study the properties of wheat starches and to find out the effect of variety and conditions of growth on these properties. For the most part we have to be guided by previous studies which were carried out on starches derived from different species of plants. However, these studies should give clues to those properties in which variations might be expected.

Variation in Physical Properties of Starches.

From the standpoint of susceptibility to enzyme attack, the physical properties of the starch grains may be just as important as their chemical nature, either because of a direct physical effect or because physical differences may indicate differences in composition. Reichert's monumental work (62) is undoubtedly the outstanding research along this line. He was able to establish differences in form, appearance, and size, on the physical side, and differences in temperature of gelatinization, polariscopic properties, and staining reactions, which are probably partly chemical and partly physical, and differences in the chemical behaviour towards various reagents.

The swelling of starch grains has received some attention. Alsberg (5) noted that potato starch differed from most starches in that it disintegrated on boiling. Huss (33) used stains in the study of swelling. He showed that starch grains, changed by pressure, heating, or chemical action, will absorb eosin, congo red, phenol red and a number of other dyes which will not penetrate the uninjured grains.

Marked variation in the size of the granules from different sources has been noticed. Alsberg (5) points out that there is little correlation between the original size of the granule and the size after swelling, owing to the many

variables affecting both measurements. The multiplicity of factors affecting granule size affords an explanation of the results obtained by Grewe and Bailey (29) who were unable to demonstrate any relation between size of grains and the quantity of maltose produced by diastatic action in flour. Buchanan and Naudain (17, 53), however, have reported that strong flours contain a higher percentage of small granules than weak flours.

The gelatinization temperature has been investigated by several workers and differences have been found not only between species but even within varieties. Fernbach and Wolff (25) found starch from immature peas to be different in this respect from that of a mature sample of the same variety. La Wall and Graves (37) found differences within varieties of peas and beans. Varieties of maize were found by Dox and Roark (22) to vary in this property. Alsberg and Rask (2), using a viscosity method, found that gelatinization of wheat and corn starch took place over a range of temperature differing for the two starches.

Rask and Alsberg (60) found significant variations in the viscosity of starch obtained from eleven different flours. Samec (64) reports differences in the viscosity of potato, wheat, corn and rice starches.

Differences have also been found in osmotic pressure (64), specific gravity (86) and other physical properties, but the foregoing review is sufficient to show the variability of the physical nature of starch within one variety as well as between species.

Variation in Composition of Starch.

We now know that starch grains are not composed entirely of carbohydrate material, and that even the carbohydrate portion is not pure starch. Phosphorus, probably in organic combination, is a constituent of starch grains and varies in quantity from species to species. Samec (64) reports variation in the phosphorus content. Malfitano (48) was able to find SiO_2 in starch granules. Lately the fat content of starch has been attracting attention. Taylor and Nelson (81) report its occurrence in corn starch. It has also been found (8) in rice, and variations in amount in different samples have been noted.

Ling and Nanji (42) report the presence of hemicellulose in some starches but not in others. Schryver and Thomas (67) found a variation of from 0 to 4% in the hemicellulose content of six starches examined. Clayson and Schryver (19) isolated a hemicellulose from wheat flour and found it identical with that from wheat starch.

There is considerable evidence that starch itself is not a homogeneous substance but is composed of at least two fractions. These have been termed (1) starch cellulose, amylopectin or α-amylose and (2) granulose, amylose or β-amylose. The first set of terms refers usually to the outer portion of the granule and the second to the inner. It must be remembered that, in the present state of our knowledge, the substances to which these terms refer are not very accurately defined, and that some workers (9) hold that the amylopectin is scattered throughout the grain. Nearly a hundred years ago Guibort (30) called attention to the difference between the outer coating and

the inner soluble material in the granule. Since that time a great deal of work has been done on this phase of starch chemistry. Bourquelot (14) in 1887 concluded that starch granules are composed of a mixture of carbohydrates. Meyer (51) fully described the preparation of α- and β-amylose. Samec and Haerdtl (64) found that the quantities of amylopectin and amylose in a number of starches, including wheat, maize, rice, and potato, varied considerably. To this they attribute the variation which they found in water content, viscosity and resistance to diastatic action, since Samec and other co-workers (66) were able to show considerable differences in the physical and chemical properties of the amylopectins. Chodat (18) holds that the degree of polymerization of the two fractions may vary from sample to sample and that from this there result differences in behaviour towards diastase. Taylor and Iddles (82) carried out the separation of α - and β -amylose from a number of common starches and found marked differences in the properties, notably that α-amylose is very slowly hydrolyzed by 10%HC1, while β-amylose hydrolyzes readily to a reducing substance. They also found variations in the amounts present in the starches studied. There were differences between samples of a single species, though not so large as between different species. It is still an open question whether the difference between the two fractions is chemical as a difference in degree of polymerization, or physical, as a difference in degree of hydration. However, the fact remains that starches from different sources vary in practically all the properties that have been studied, and that variations are to be found, though usually to a lesser degree, between samples of starch from the same species of plant. It is not surprising, therefore, that many investigators have found similar variations in the reaction to diastase.

Variations in Resistance of Starch to Diastatic Action.

Unfortunately, much of the work on this phase of the subject has been with gelatinized starches, and the results are thus not strictly applicable to the present problem. The weight of evidence, however, seems to be in favour of the existence of a variation between starches from different species of plants Ford (27), O'Sullivan (59), and Sherman, Walker and Caldwell (72) all found no significant difference between samples from different sources, using boiled starches, but O'Sullivan noted that a much lower percentage of maltose was obtained from potato starch than from the cereal starches. On the other hand, many papers could be cited, where the opposite results were obtained. The investigations of Levberg (38, 39) and of Lintner (43, 44) in the latter part of the last century fall in this group. Nagao (52) found oat and barley starches were digested more rapidly than wheat or rye starches. Lintner and Baur (45) were of the opinion that rye and barley starches varied in their resistance to diastatic action.

In the present paper, however, the chief interest lies in the evidence for variation in the resistance of the starch in different samples of wheat flour. In a microscopic study of germinating wheat, Whymper (87) showed that there was a variation in the ease with which granules of different sizes were attacked. In 1909 Humphries and Simpson (32) brought forward evidence

to show that the rate at which the starch granules are attacked, and not the activity of the enzyme, is the prime factor that determines the gas-making capacity. Rumsey (63), working with one flour and two samples of wheat starch, obtained 247.0, 157.8 and 215.2 mg. of maltose, respectively, produced by the action of 1 gm. of malt extract. Collatz (20) added varying amounts of malt flour to seven samples of flour and one sample of wheat starch. He found that not only were there differences in the amount of reducing sugar produced by the same amount of diastase but that the flours did not maintain the same order as the amount of malt flour was increased. He says, "In general but for one exception the weaker flours produce less reducing sugars than do the stronger when digested with the same amount of malt flour.....From the data presented, it would seem that the starch of the strong flours was generally more easily converted than that of weak flours." In a later paper, with O.C. Racke (21), he was able to demonstrate that there were substantial differences in the amount of reducing sugars produced by malt extracts in doughs from different flours. Mangels (49) recently reported the results of a study of the factors affecting the diastatic activity of flour. He determined diastatic activity by Rumsey's method, and then used commercial starch as the substrate for the action of a cold-water extract of three of his flours and obtained only slight variations in the amount of maltose produced. The differences in the results of the Rumsey determination, therefore, were due principally to variations in the resistance of the starch. He also subjected three wheat starches to the action of a cold-water extract of Marquis flour, and obtained 44.2, 10.1, and 182.0 mg. of maltose, respectively.

Hermano and Rask (31) working with wheat starches, prepared by the method of Rask and Alsberg (60), and malt diastase, were able to show marked differences between samples of starch obtained from different varieties of wheat grown at different places. It will be seen that the evidence points to the existence of demonstrable differences in the resistance of the starch in different samples of flour to the action of diastase.

Variation in the Action of Diastase.

There is abundant evidence that diastatic enzymes from different sources vary enormously. It is only necessary to mention two studies, which are typical of the bulk of this class of work. Wohlgemuth (88) found that the diastase from various animal sources showed a variation in relative diastatic values of from 10 to 468.8 when a 0.1% starch solution was used as the substrate. Oshima and Itaya (57), working with vegetable diastases, obtained values in amylo-saccharifying units varying from 0 for ungerminated oats and barley to 21,000 for ungerminated soy beans. These authors also studied the variation in the amylo-liquifying and amylo-dixtrinizing values. They found that not only did the magnitude of each of these vary but that the ratio between them varied. Their results are in agreement with those of Effront (24) who found that he was able to distinguish between diastatic enzymes from different

sources on the basis of the relation of liquifying to sugar-producing power. Numerous other investigators, notably Sherman and his co-workers (70,71) have drawn attention to this dual function of diastase.

The apparent existence of two separate functions has led to the theory that there are two components of diastase or else two distinct but associated enzymes. This was suggested fifty years ago by Maercker (47) and the suggestion has been repeated from time to time since then. Bourquelot (12) is sometimes credited with being the first to propose the theory, but Maercker's publication preceded his by nine years. Until recent years, however, attempts to separate the two enzymes have been largely fruitless Ohlsson (55, 56) in 1922, and in a more comprehensive paper in 1926, described a method for their separation. The "saccharogen-amylase" is prepared by the destruction of the other component; this is accomplished by allowing the malt solution to stand for 15 minutes at 0° C. and a pH of 3.3. The "dextrinogen-amylase" is prepared by taking advantage of the instability of the saccharogen-amylase at a temperature of 70°C.

The foregoing discussion shows clearly that variations in sugar production from any starch may be caused by qualitative differences in the enzyme present. That variations may occur as a result of quantitative differences in the enzyme content is a commonplace, and the literature which could be cited in support of it is voluminous.

Very little of the foregoing work has been done with wheat diastase however, so that direct evidence on the problem of the variation, whether qualitative or quantitative, in its sugar-producing power is rather limited. It has already been noted that Mangels (49) found slight differences in the activity of the cold-water extracts of three samples of wheat flour. Thatcher and Koch (85) using several procedures for the water extraction, found a variation in the amylolytic power of the diastase extracted from different grades of flour.

Experimental

The Problem

The literature reviewed in the previous section shows clearly that we may expect variations in the physical and chemical properties of the starch in different samples of wheat flour and a consequent variation in the resistance to diastatic attack. We may also expect to find variations in the sugar-producing power of the diastase, owing to either qualitative or quantitative differences in the enzyme content. It is evident, then, that the values for diastatic activity obtained by Rumsey's method are the resultant of two groups of variable factors. It is admitted that from the point of view of the baker the quantity of maltose produced is the important factor, regardless of whether it is influenced most by the activity of the enzyme or the resistance of the starch. In a study of the effect of environment on wheat quality, however, it is highly desirable that the two groups of factors be studied separately,

as it is reasonable to assume the possibility of the resistance of the starch and the activity of the enzyme being influenced differently by environmental conditions.

For a preliminary study on a large scale, such as the one at present in progress at the University of Alberta, it is only necessary to measure one of the sets of factors affecting diastatic activity, and the other can be evaluated by comparison of the results so obtained with the results of the Rumsey determination. It is fully realized that each group of factors is quite complex, and studies with a view to estimating the importance of each of the individual factors in both groups will be undertaken as soon as possible.

In the present investigation it was first necessary to decide whether the starch resistance or the activity of the enzyme could be more easily and accurately measured. Rumsey's review of the literature bearing on this point was sufficiently convincing to warrant the choice of the former as the most satisfactory line of attack. He shows that it is difficult to obtain a satisfactory substrate. It is possible that this might have been overcome by the preparation of a large quantity of wheat starch, but the extreme difficulty of obtaining a quantitative extraction of the diastase is a grave objection which cannot be so easily avoided. The experiments, therefore, were carried out with a view to finding a method which would measure the resistance of the starch and which would be suitable for routine use on a large number of samples.

It appeared that this could be most effectively accomplished by the adoption of a method in which the natural diastase is rendered inactive and the starch is subjected to the action of a constant quantity of added diastase. Both Collatz (20) and Rumsey (63) carried out experiments on the resistance of starch by adding malt diastase to a flour suspension, determining the total sugar produced and correcting for the original diastatic activity of the flour. While no criticism of this procedure as a method for demonstrating the fact of variation in the substrate is intended, it did not appear to the author to be a satisfactory method for quantitative estimation of the resistance of the starch, where relative results are of prime importance. The possibility of qualitative differences in the diastase in different samples and the lack of any knowledge of the combined effect of diastase from different sources, made it seem advisable to get rid of the natural diastase.

Preliminary Experiments

The preliminary experiments were entirely of a qualitative nature. In the first group the effect of certain volatile substances was tried, with the idea that the flour could be treated and the added substance allowed to evaporate, leaving the flour diastase inactive. These experiments were not very extensive, nor was any great hope entertained for their successful outcome, as in the present state of our knowledge the finding of a suitable substance would be largely a matter of chance. Two compounds were tried unsuccessfully—chloroform and carbon disulphide. In each case marked reduction of Fehling's solution was noted after digestion of the flour. In so far as the chloroform is

concerned, this result agrees with the findings of Sherman and Wayman (73). In the same paper, these authors report the inhibiting effect of formaldehyde on diastase. In view of this, a sample of flour was doughed with formalin and allowed to stand over night. The author was acquainted with the denaturing effect of formaldehyde on proteins, but even so he was quite unprepared to find the tough rubbery mass which resulted. Apart from this effect on the proteins, it is doubtful if formalin would have been a suitable substance, because of its action on starch, giving a compound which does not show the iodine reaction (65).

The extraction of the starch by the excellent method of Rask and Alsberg (60) was tried but was deemed unsuitable for several reasons. It is too laborious to be suitable for routine determinations. In addition there is a slight amount of residual diastase found in the prepared starch and the washing with ether in the course of the preparation is a disadvantage, because of the effect of ether extraction on starch resistance.

The next series of experiments was designed to test the efficiency of inhibiting agents which could be added in solution and the excess of which could be washed out. Sodium tungstate was the first reagent tried, using the procedure for inactivation recommended by Rumsey and getting rid of the excess by washing and centrifuging three times with distilled water. After the third washing, the sample was allowed to digest for one hour at room temperature. A qualitative test with Fehling's solution showed no reduction. This procedure appeared so promising that quantitative experiments were started to determine its suitability.

Inactivation of Natural Diastase.

The procedure followed was similar to that in the qualitative experiments. Ten grams of flour was suspended in approximately 75 cc. of water in a centrifuge tube and immediately inactivated. The suspension was made slightly alkaline, 3 cc. of 15% sodium tungstate was run in and sulphuric acid was added drop by drop until flocculation took place. The tube was then filled to capacity with distilled water and centrifuged. The supernatant liquid was decanted and replaced with distilled water and the mass of flour in the bottom of the tube was stirred up. The tube was then shaken to ensure thorough washing and was recentrifuged. This washing procedure was repeated three times. After the last decantation the flour was transferred to a 200-cc. Kohlrausch flask and suspended in 100 cc. of citrate buffer solution (pH. 4.7 approx.)[1]

The suspension was allowed to stand for one hour at 27°C. The sample was again subjected to treatment with sodium tungstate, centrifuged, and an aliquot taken for determination of reducing sugars by the method of Shaffer and Hartman (69), which was used throughout these experiments.

[1]*The use of buffer solution instead of distilled water is to make the procedure analogous to our modification of Rumsey's method for diastatic activity. A full discussion of the reasons for adopting this procedure will be found in the section dealing with these modifications.*

Some difficulty was experienced owing to the formation of a heavy foam in making the original suspension. On centrifuging, this compacted into a solid layer on top of the liquid, and effectively prevented a clean separation by ordinary decantation. This difficulty was obviated by two means. It was found that if three drops of toluene, which has no effect on diastase (73), are added to the water in the tube before shaking, the amount of foam is much reduced. The removal of the supernatant liquid, after centrifuging, is accomplished by aspirating it out through a small glass tube. It was not considered safe to use caprylic alcohol as an agent for cutting the foam because of a possible effect on the diastase.

It was noticed that after the second washing there was a tendency for the supernatant liquid to become cloudy. The addition of about 15 cc. of the citrate buffer solution to the wash water helps to remedy this condition.

TABLE I

ACTIVITY OF NATURAL DIASTASE AFTER INACTIVATION AND WASHING

Sample	Thiosulphate titration in cc.
Shaffer & Hartmann blank	13.80
Flour No. 1	13.68 13.65
Flour No. 2	13.80 13.80
Flour No. 3	13.85 13.82 13.80
Flour No. 4	13.70 13.70

In Table I it may be seen that the results of all determinations agree with the blank on the reagents within the experimental error of the method. This, of course, means that there were no reducing substances present in the aliquot. The reducing sugars originally present in the flour had all been removed by the washing procedure. The natural diastase of the flour had been completely inactivated and had remained so under the conditions under which the digestion with added diastase would be carried out. The entire absence of reducing sugars is very fortunate, because it obviates the necessity of running a control sample with every determination.

The next step was to see whether or not the washing procedure had completely removed the inactivating agent. When taka-diastase was added to a washed sample, digestion proceeded in a perfectly normal manner. Parke-Davis taka-diastase was selected as the enzyme to be used in these experiments as

it is readily available in a fair state of purity. The validity of its use in testing the resistance will be dealt with in a later section dealing with the comparison of flour diastase and taka-diastase.

Procedure for Second Inactivation.

Owing to the presence of the buffer solution, difficulty was experienced in following Rumsey's directions for the use of sodium tungstate after digestion. Since the material to be inactivated has a constant acidity it was deemed allowable to use a fixed procedure for carrying out this step in the process. Experiments were, therefore, performed to determine a procedure which would give satisfactory inactivation and clarification.

A series of 10-gm. samples of flour was freed from natural diastase and transferred to Kohlrausch flasks. To each was added 0.025 gm. of taka-diastase and the sample was immediately inactivated, using 3 cc. of 15% sodium tungstate solution as directed by Rumsey, but varying the amount of N/1 NaOH and H_2SO_4 (1:1) used. Table II gives the thiosulphate titration, after one hour digestion at 27°C. and the efficiency of clarification.

TABLE II

EFFECT OF VARYING AMOUNTS OF ALKALI AND ACID ON INACTIVATION AND CLARIFICATION

N/1 NaOH, in cc.	H_2SO_4 in cc.	Thiosulphate titration, in cc.	Clarification
1	0.6	12.50	good
2	0.6	12.01	good
3	0.6	12.37	very good
4	0.6	12.47	very good
1	1.0	12.52	very good
2	1.0	12.61	very good
3	1.0	12.73	excellent
4	1.0	12.36	excellent
1	1.3	12.61	excellent
2	1.3	12.60	excellent
3	1.3	12.70	good
4	1.3	12.64	good
*Blank		12.70	

**For reducing substances in diastase.*

It will be seen that the best inactivation was obtained by the use of 3 cc. of NaOH and either 1.0 or 1.3 cc. of the acid-water mixture. However, the clarification using 1.0 cc. of acid was markedly better than that when 1.3 cc. of acid was used. A further test using 3 cc. of NaOH and 1 cc. of acid was therefore carried out. The procedure was the same as before but only the one amount of acid and alkali was used. The quantity of taka-diastase was 0.03 gm. instead of 0.025 as in the previous experiment. This accounts for the smaller titration for the blank. Four different samples of flour were used. The results are given in Table III.

TABLE III

TEST OF THE EFFICIENCY OF PROCEDURE FOR INACTIVATION AND CLARIFICATION

Sample	Thiosulphate titration in cc.	Clarification
Flour No. 1	12.52	Excellent
Flour No. 2	12.38	Excellent
Flour No. 3	12.50	Very good
Flour No. 4	12.38	Excellent
Blank	12.52	

All these titrations agree with the blank within the limits of experimental error. All subsequent inactivations in samples containing buffer solution were therefore carried out by adding in succession 3 cc. of N/1 NaOH, 3cc. of 15% sodium tungstate solution, and 1 cc. of H_2SO_4 (1:1).

Description of Flours used.

Before a definite procedure could be adopted it was necessary to test the effect of the variation of several factors on the results obtained. A description of the three flours used in these experiments follows:

Flour A—Milled in the laboratory experimental mill from hard southern-Alberta Marquis.

Flour B—A commercial patent.

Flour C—A composite flour composed of a mixture of residues of samples of Red Bobs 222 and Marquis milled in the laboratory experimental mill.

Effect of Variation in the Amount of Diastase Added on the Quantity of Reducing Sugar Produced.

It was necessary to get an idea of the amount of reducing sugars that might be expected to result from the digestion with varying amounts of diastase and to establish the type of relationship between amount of enzyme and sugar production.

Ten-gram samples of Flour A (dry weight) were treated according to the procedure outlined in the previous section and digested for one hour at 27°C. with varying amounts of taka-diastase. Blanks containing the same quantities of diastase and buffer solution, but no flour, were run concurrently. The amounts of reducing sugars obtained from the flour were corrected for the amounts found in the blanks. The results are given in Table IV and graphically in Fig. 1.

TABLE IV

EFFECT OF AMOUNT OF TAKA-DIASTASE ADDED ON THE AMOUNT OF SUGAR PRODUCED

Weight of taka-diastase in gm.	Reducing sugar as maltose per 10 gm. of dry flour, in mg.
0.01	73.
0.03	111.
0.05	174.
0.075	214.
0.105	239.
0.15	287.
0.25	372.

As was to be expected, the amount of maltose produced increased with increasing quantity of diastase. This is the common behaviour which has been noted by practically every worker in the field, one of the earliest being Schwarzer (68), in 1870.

In 1876 Kjeldahl (35) propounded his "law of proportionality", which holds that the amount of maltose formed is directly proportional to the amount of amylase present, as long as digestion is not more than 40% complete. This

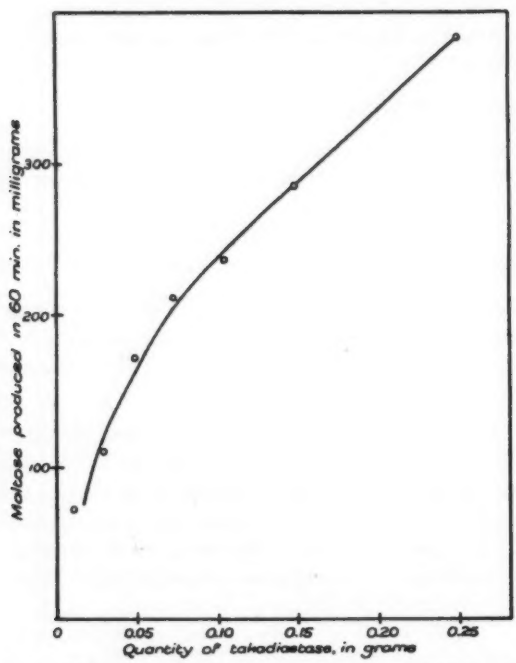

FIG. 1. *Effect of Quantity of Diastase on Amount of Maltose Produced from Ten Grams of Flour (Dry Weight).*

law has received considerable support, including evidence put forward by Ford (26). Its general validity has been questioned, however, by several workers. Ling (40) found that it does not hold for air-dry malt, and Macquaire (46), working with pancreatic diastase, found that there is a relative decrease in the amount of sugar formed per unit of diastase when the amount of diastase is increased.

The amounts of sugar formed in the present series of experiments fall well within the limit of application of Kjeldahl's law. However, it will be noticed that the same increases in the amount of diastase do not give equal increases in the reducing sugar produced, in all parts of the curve (Fig. 1). Collatz (20) obtained similar curves by the addition of varying quantities of malt flour.

The significance of the curve for the present purpose is that it gives information on which the choice of a quantity of diastase can be based. It is desirable to choose a quantity of diastase from a linear portion of the curve, that is, either less than 0.05 gm. or more than 0.1 gm. of taka-diastase. It was decided to use the lower range, since the quantities of sugar are more easily handled by the Shaffer and Hartman method. The objection that, in this part of the curve, small variations in the amount of diastase added will give relatively large differences in the amount of reducing sugar, is overcome by the adoption of an accurate technique for measuring out the diastase. A suspension is prepared and aliquots of this used for digestion.

Effect of Time of Digestion on the Amount of Reducing Sugar Produced.

Osterhout in 1918 (59) pointed out the fallacy of measuring the relative rates of biological processes by taking the amount of substance produced in a given time as a measure of that rate. He quite correctly argues that the only true measure of rate where the reaction follows the law for a reaction of the first order is the length of time necessary to produce a given amount. However, in many cases this procedure is most inconvenient, and it is not at all suited to the determination under discussion.

It should be noted, moreover, that Osterhout's calculations are based on the law for a first order reaction. Recently Eadie (23) has found that this formula cannot be applied to diastatic action and that the velocity curve approximates more closely to the formula:

$$v = a + b \log c$$

in which v is velocity, c is concentration of substrate, and a and b are constants.

Brown and Glendinning (16), using a 3% starch solution, concluded that the amount of hydrolysis up to 36% is very nearly a linear function of time, and beyond that point it is approximately logarithmic.

A study of the curves put forward by Osterhout in favour of his contention shows that in the earlier stages of any reaction, while the quantity curves are still rising sharply, the difference in the result obtained by the two methods is

not great. It was decided, therefore, to determine the effect of time of digestion on the amount of sugar produced in order to find a procedure which would not be open to criticism on the basis of Osterhout's findings.

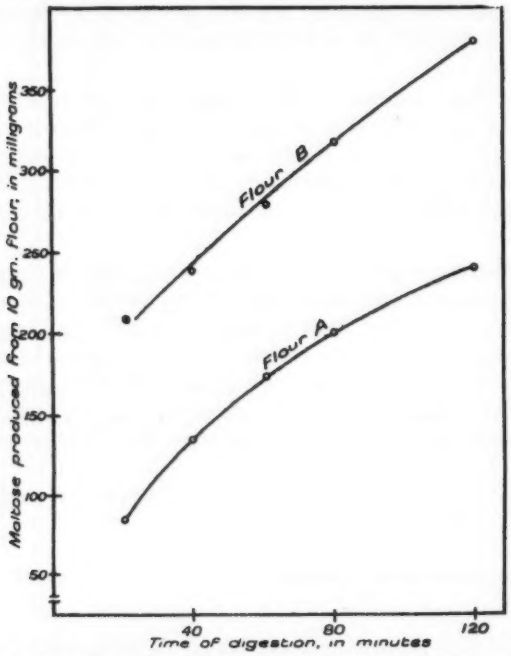

FIG. 2. *Effect of Time of Digestion on the Amount of Maltose Produced by 0.05 gm. Taka-diastase from 10 gm. Flour.*

Ten-gram samples (dry weight) of flour were used in all these experiments. The flour was inactivated as previously outlined and digested with taka-diastase for varying lengths of time and the results corrected for the taka-diastase blank. The results of the determination of the blank are rather interesting in themselves and a discussion of these will be found at the end of this section. In the first two series 0.05 gm. of taka-diastase was used. The results of these are given in Table V and Fig. 2.

TABLE V

EFFECT OF TIME ON THE AMOUNT OF REDUCING SUGAR PRODUCED BY 0.05 GM. TAKA-DIASTASE ACTING ON 10 GM. SAMPLES OF FLOUR

Time of digestion in min.	Reducing sugar as maltose in mg.		Ratio B/A
	Flour A	Flour B	
20	87	210	2.42
40	134	237	1.78
60	174	280	1.61
80	203	318	1.57
120	240	385	1.61

These data are very similar to those obtained by Rumsey (63) and Collatz (20). An examination of the curves (Fig. 2) shows that both of them, and particularly that for flour A, are from that portion of the whole time curve where the direction is changing. In order to bring the values into the approximately linear portion of the curve it would be necessary to digest for only 20 minutes if 0.05 gm. of diastase is to be used. It is not practical for routine purposes to use a digestion time of much less than 60 minutes. With short digestions, slight, unavoidable variations in the time may affect the result to an appreciable degree. On the other hand, if the time is extended, the number of samples which can be handled in one day may be seriously reduced.

The ratios between the two sets of values given in Table V are rather interesting. For the reason stated above, too much reliance cannot be place on the results of the 20-minute digestion. The other ratios, and particularly the last three, are suprisingly constant. They constitute an indication that Osterhout's criticism of the method of measuring relative rates by taking the amount produced in a given time may not apply in this case.

Because of the fact that most of the values did not fall on the linear portion of the curve, it appeared that further information was required before a definite procedure could be adopted. Consequently, a similar series of experiments was carried out using 0.03 gm. taka-diastase instead of 0.05 gm. The results of these experiments are given in Table VI and Fig. 3.

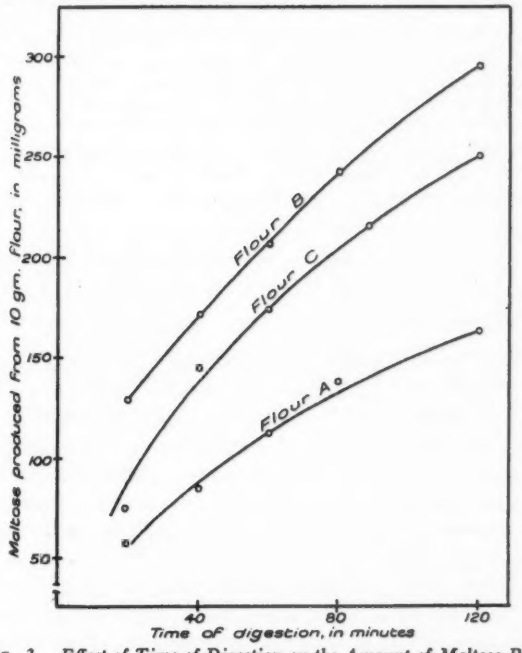

FIG. 3. *Effect of Time of Digestion on the Amount of Maltose Produced by 0.03 gm. Flour from 10 gm. Flour.*

TABLE VI

EFFECT OF TIME ON THE AMOUNT OF REDUCING SUGAR PRODUCED BY 0.03 GM. TAKA-DIASTASE ACTING ON 10 GM. SAMPLES OF FLOUR

Time of digestion in min.	Reducing sugars as maltose in mg.			Ratios	
	Flour A	Flour B	Flour C	B/A	C/A
20	56	129	75	2.03	1.34
40	83	171	145	2.05	1.74
60	111	206	174	1.86	1.56
80	137	243	206	1.81	1.50
120	160	295	250	1.85	1.56

The curves obtained from this series are very similar to those from the previous experiment. The ratios as before show remarkable constancy for digestion times of 60 minutes or more. These results show that 60 minutes digestion is just as satisfactory as a longer period. In order to decide whether the procedure of measuring the amount of maltose produced in a given time is reliable, it is necessary to compare the data in Table VI with those given in Table V. This can be most easily done by examining the ratios obtained when the amount of sugar produced by 0.05 gm. diastase is divided by the amount produced by 0.03 gm. diastase. These ratios are given in Table VII.

TABLE VII

EFFECT OF TIME OF DIGESTION ON THE RATIO OF SUGAR PRODUCED BY 0.05 GM. TAKA-DIASTASE TO THE AMOUNT PRODUCED BY 0.03 GM. TAKA-DIASTASE

Time of digestion in min.	Ratio of sugar produced by 0.05 gm. and 0.03 gm. taka-diastase	
	Flour A.	Flour B
20	1.55	1.62
40	1.61	1.39
60	1.56	1.35
80	1.48	1.32
120	1.49	1.31

These ratios are also quite constant, and allowing for the difficulty of exact determination for a 20 minute period we can say that the ratio is constant throughout the range studied. Not only is this true but the ratios have values which might be expected from the amounts of taka-diastase used. If the relation between quantity of diastase and amount of sugar produced were a linear function this ratio would be $5/3 = 1.67$. However, we have seen that the relationship is approximately logarithmic in type, consequently the value of the ratio will be slightly lower. This would bring it into agreement with the ratios obtained from the series with Flour A. The fact that the ratios obtained by the use of Flour B are lower can probably be attributed to the fact that the starch of Flour B is more easily converted. While experiments on the effect of quantity of diastase were not carried out with Flour B, we would

expect that the curve would show pronounced curvature at lower values than those in the curve for Flour A. The more pronounced the curvature, the lower will be the ratio between the sugars produced by any two quantities of diastase.

The relative rate of hydrolysis with the two quantities of diastase using Osterhout's method (the time necessary to produce a given amount of sugar) was determined. Table VIII shows the ratios which were calculated from the values taken from the common portions of the curves.

TABLE VIII

RATIOS BETWEEN TIMES NECESSARY TO PRODUCE A GIVEN AMOUNT OF SUGAR, USING 0.03 AND 0.05 GM. DIASTASE

Amount of sugar, in mg.	Ratios	
	Flour A	Flour B
100	2.04	—
150	2.10	—
250	—	1.93
290	—	1.85

It will be seen that these ratios are similar to those obtained by the other method, in that they are constant for each flour and that those for Flour B are lower than those for Flour A. However, the values of the ratios are peculiar. It was pointed out above that the value of any such ratio should be 1.67 or less if it is to be a reliable index of the relative activity of the two amounts of added diastase. It is inconceivable that any ratio above that value can give a correct picture of the situation. The statement that the measurement of relative rate in this instance can be more accurately carried out by determining the amount of sugar produced in a given time than by determining the time necessary to produce a given amount of sugar appears to be amply justified.

After consideration of the results reported in this section, it was tentatively decided to use 10-gram samples and to digest for 60 minutes with 0.03 gm. taka-diastase. The final fixing of the procedure would depend on the effect of the size of the sample on the amount of sugar produced.

The Taka-diastase Blank.

In connection with the experiments just reported the amount of sugar produced by the auto-digestion of taka-diastase for varying lengths of time was determined. The diastase was suspended in 100 cc. of the buffer solution and allowed to digest at 27°C. for the lengths of time specified. The enzyme was then inactivated by the sodium tungstate procedure and the reducing sugars determined on an aliquot. The results of two such series are reported in Table IX.

TABLE IX

VARIATION IN THE TAKA-DIASTASE BLANK WITH TIME OF DIGESTION

Time of digestion, in min.	Reducing sugar as maltose in mg.	
	0.05 gm. taka-diastase	0.10 gm. taka-diastase
0	37	80
20	34	79
40	35	79
60	38	80
80	35	78

It can be readily seen that the sugar is present in the taka-diastase before digestion, also that taka-diastase is composed of practically 80% of reducing substance calculated as maltose. It is hardly probable that the reducing substances are all in that form but they must make up a considerable proportion of the weight of the prepared enzyme.[1]

Effect of Concentration of Substrate on Amount of Reducing Sugar Produced.

In this series of experiments the samples were inactivated as usual and digested for one hour with 0.03 gm. taka-diastase. The results of the experiment together with the ratios of the values for the different flours are given in Table X, and the graphs drawn from the data are to be found in Fig. 4.

TABLE X

EFFECT OF CONCENTRATION OF SUBSTRATE ON THE AMOUNT OF REDUCING SUGAR PRODUCED BY 0.03 GM. TAKA-DIASTASE

Dry weight of sample, in gm.	Reducing sugar as maltose in mg.			Ratios	
	Flour A	Flour B	Flour C	B/A	C/A
8	95	174	151	1.83	1.59
10	111	206	174	1.85	1.56
12	130	253	199	1.94	1.53
13	139	265	213	1.90	1.52

The results of these experiments are rather surprising, as it was fully expected that, in view of the excess of substrate, the amount of sugar produced would not vary more than a few milligrams.

The variation is rather difficult to explain without further research. One possible explanation is that the real substrate for the saccharogenic enzyme is liquefied starch and that the production of this is conditioned by the amount of raw starch. It was thought at first that the variation might be due to a dilution effect since the same amount of buffer solution (100 cc.) was used

[1]*It has since been ascertained that the Parke Davis Co. use maltose to dilute their diastatic preparations.*

for digestion in each case. However, the results obtained by Swanson and Calvin (79) make this explanation improbable. Fortunately, the ratios of the amounts of sugar produced by different flours at each concentration of substrate show that it makes little difference which of these concentrations are selected, as long as the same amount is used in every determination. It should be remembered, however, that equal amounts of flour do not necessarily mean equal concentrations of starch. The two chief causes of variation in the percentage of starch in flour are variation in the moisture content and variation in the percentage of protein. We have already eliminated the first

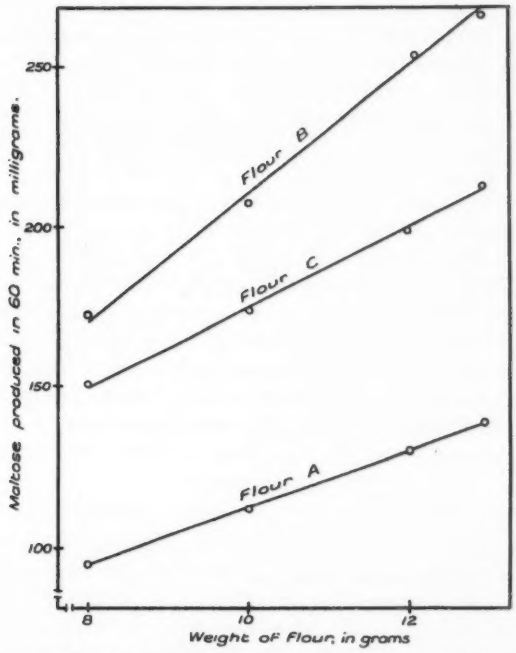

FIG. 4. *Effect of Concentration of Substrate on Amount of Maltose Produced by 0.03 gm. Taka-diastase.*

of these by adopting the dry basis for the weight of all samples. There are two methods of compensating for variation in the amount of protein. We can weigh out the sample on a constant protein basis or we can correct the results of the determinations to bring them to a constant protein basis. This latter procedure is allowable since the curves obtained from the data in Table X are approximately straight lines. It was decided to adopt 13.5% as the standard protein content, principally because the tables prepared by Shollenberger and Coleman (75) for the conversion of crude protein content to a uniform moisture basis could be used equally well for converting the amount of sugar

produced to a uniform protein content basis. It will depend, of course, on the particular experiment whether it will be desirable or necessary to make this correction.

In view of the unexpected result of this experiment, it was considered desirable to determine the effect of variation in substrate concentration, using 0.05 gm. taka-diastase, in order to ascertain whether a similar curve would be obtained. The determinations were carried out in the same manner as in the previous experiment with the exception that a different quantity of diastase was used. The results are given in Table XI.

TABLE XI

EFFECT OF CONCENTRATION OF SUBSTRATE ON THE AMOUNT OF REDUCING SUGAR PRODUCED BY 0.05 GM. TAKA-DIASTASE (FLOUR A)

Dry weight of samples in gm.	Reducing sugar as maltose in mg.	Ratio between sugar produced by 0.05 gm. and 0.03 gm. taka-diastase
8	140	1.48
10	174	1.54
12	201	1.56
13	207	1.48

These results gave the same type of curve as those drawn from the results in Table X. The ratios shown in the table are practically constant and of the same value as those obtained in the experiment on effect of time (Table VII). This was taken as further proof of the statement previously made that the size of sample used is immaterial, as long as the same amount is used in every determination.

METHOD ADOPTED

Ten grams of flour (dry basis) is weighed into a 100-cc. centrifuge tube and a little distilled water poured in on top of it. Three drops of toluene are added and the tube filled about three-quarters full with distilled water. The flour is brought into suspension by shaking. The suspension is then made neutral or slightly alkaline with N/1 sodium hydroxide and the tube shaken to ensure thorough mixing. Three cubic centimetres of 15% sodium tungstate is added, the tube shaken again, and a sufficient quantity of sulphuric acid (1:1) to bring about flocculation is added drop by drop. The tube is shaken again and filled up with water. The suspension is then centrifuged for 4 min. at stop No. 6 (size 2, International centrifuge). The supernatant liquid is removed by aspiration through a small glass tube. About 15 cc. of the buffer solution (pH. 4.7 approx.) is poured in and the mass of flour is loosened from the bottom of the tube with a stirring rod. Distilled water is poured in until the tube is about three-quarters full when the tube is shaken to bring the flour into suspension. The tube is then filled with water and centrifuged as before. This washing procedure is carried out three times. After the last washing, the flour is transferred to a 200-cc. Kohlrausch or other wide-necked volumetric flask, using 95 cc. of buffer solution, at such

a temperature that the temperature of the suspension in the flask will be approximately 27°C. The flask is then put in a constant-temperature water-bath set at exactly 27°C. and allowed to remain for about 15 min. to equalize the temperature.

The buffer used is one of Sörensen's citrate-HCl buffers (90). The citrate solution is made up of 21.008 gm. crystalline citric acid plus 200 cc. of N/1 NaOH per litre. The particular buffer used contains 4 parts of citrate solution to 1 part of N/10 HCl, and it is used in a concentration of 1 part of buffer solution to 3 parts of distilled water.

While the suspensions are being brought to temperature a suspension of taka-diastase in buffer solution is made up so that 5 cc. of suspension contain 0.03 gm. taka-diastase. The 5 cc. of this suspension is pipetted immediately into each flask and the time is noted. The digestion is allowed to proceed for exactly 60 min. and the flasks are shaken at intervals during this time. As soon as digestion is completed the suspension is diluted to about 175 cc. with distilled water and inactivated by the addition of 3 cc. of N/1 NaOH, 3 cc. of 15% sodium tungstate and 1 cc. of sulphuric acid (1:1) in succesion. The suspension is then made up to volume (200 cc.) and 7 cc. more water added to compensate for the volume of the flour. The flask is shaken till the contents are thoroughly mixed. The sample is then transferred to a centrifuge bottle and centrifuged for 4 min. at stop No. 10 (size 2, International centrifuge). An aliquot (usually 50 cc.) is taken for the determination of reducing sugars by the Shaffer and Hartmann method. Where results reported in the following pages are tabulated as "starch resistance" the numerical values for maltose are converted to their reciprocals, multiplied by 10,000 to avoid decimals. This makes them vary in the same sense as the actual resistance of the starch.

Comparison of Taka-diastase and Extracted Flour Diastase.

It was thought to be desirable to compare the results of the method adopted with those obtained by a similar method, using extracted flour diastase. Accordingly the starch resistance of a series of seven flours (the three experimental flours and four others) was determined by the method adopted. The sugar produced by extracted flour diastase was determined on the same flours. As it was possible to run these determinations on only three flours at one time, the flours were grouped in three series, Flour A being included in each one.

The diastase was extracted from 300 gm. of flour by 1500 cc. of buffer solution for 1.5 hours at room temperature and the extract was centrifuged. The inactivated flour to be digested with flour diastase was suspended in 50 cc. of buffer solution, and 50 cc. of the flour extract—representing the equivalent of 10 gm. of flour—was added to it. The rest of the procedure was the same as in the regular method. The amount of reducing substances in the extract was determined, using 50 cc. of flour extract and 50 cc. of buffer solution. This blank in all cases was quite high owing to the amylolytic activity during the period of extraction.

The reducing sugar produced from Flour A in the three series varied from 99 mg. to 130 mg. While the relation between the activities of the diastase extractions over this range is probably not strictly linear, it was deemed allowable to treat it as such in order to facilitate comparisons. The results of the other two series were reduced to the basis of the activity of the diastase in the first sample. The results as calculated are given in Table XII, and the values for 0.03 gm. of taka-diastase and flour diastase from 10 gm. of flour are plotted against each other in Fig. 5.

TABLE XII

REDUCING SUGAR PRODUCED BY TAKA-DIASTASE AND FLOUR DIASTASE

Sample	Reducing sugar as maltose in mg.	
	0.03 gm. taka-diastase	Flour extract from 10 gm. Flour
A	111	99
B	206	311
C	174	222
D	145	116
E	134	127
F	124	106
G	169	179

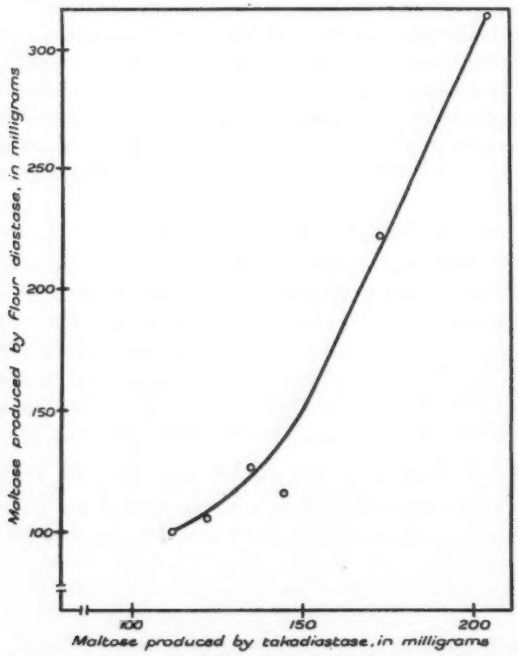

FIG. 5. *Comparison of the Amylolytic Powers of Taka-Diastase and Extracted Flour Diastase.*

It will be seen that with the exception of Flours D and E the samples fall in the same order of activity in both series, though the range is greater with the flour extract. The difference between these samples D and E is small, and considering the large possibility of error in the determination with diastase extracted from flour this irregularity cannot be regarded seriously. It seems probable that the value for Flour D with diastase extracted from flour is too low. With the exception of the point for this flour, the values plotted against each other in Fig. 5 fall very close to a smooth curve. This is a good indication that some definite relation exists between the two sets of values and consequently that the use of taka-diastase in the determination is justified. It may be possible at some later date to determine this relation exactly by running a large number of paired samples, using taka-diastase and flour diastase extracted by a more refined method.

Some Factors Affecting Diastatic Activity and the Resistance of Starch

Alsberg (6, 7) has summarized the factors affecting the diastatic activity of a flour as follows "(1) the numbers of mechanically injured starch grains it contains, (2) the ease with which its uninjured starch grains are attacked, (3) its diastatic content, and (4) the localization and character of its diastase." For the purpose of the investigations now in progress at the University of Alberta, it is more convenient to divide the factors included or implied in the above summary into two groups: (1) properties influenced by the conditions under which the wheat is grown, (2) variation in these properties owing to the milling process. Alsberg's factors 2, 3 and 4, can be included in group 1. In this group also we might include the possibility of other constituents of the flour affecting the resistance of the starch and the activity of the enzyme. Fat is one constituent that might be particularly referred to in this regard. In considering the variation in this group of factors, the possibility of fruitful research on the effect of a number of fundamental influences suggests itself. A few of these are seasonal temperature and precipitation, variety of wheat, stage of maturity at cutting, and damage, such as sprouting, rust or frost.

In the second group Alsberg makes mention in his summary of only one factor, namely, the proportion of mechanically injured grains. In his discussion, however, he brings out the fact that differences in the fineness of grinding of the flour may have a pronounced effect.

A preliminary study of a few of the points just mentioned was undertaken. As the determination of diastatic activity occupied a prominent place in these studies, it seems advisable before going on to discuss the results of the experiments, to discuss the modifications of Rumsey's method used in this laboratory.

Modification of the Method for Determining Diastatic Activity.

Rumsey found that diastatic activity varied greatly with temperature, and accordingly specified in his method that the temperature of digestion should be exactly 27°C. He also found that diastatic activity was profoundly

influenced by the acidity of the flour suspension, the maximum being reached about pH 4.7, but he did not recommend the control of this variable. Sörenson (77) points out the impossibility of obtaining comparative results at varying hydrogen-ion concentration. He suggests that the ideal procedure is to make determinations at a number of different acidities. However, it appears to the author that where this procedure is not practical, on account of the labour involved, it is desirable to make all determinations in the region of the optimum pH. The easiest method of controlling the acidity is to use a buffer solution. It was necessary, therefore, to find a buffer solution which would give the requisite pH and which was made up of substances which would neither stimulate nor depress the activity of the diastase, apart from their effect on acidity. One of Sörenson's citrate-HCl buffers (90) (citrate solution, 8 parts; HCl 2 parts) was finally chosen. In view of the fact that flour is itself a buffer, it was necessary to determine by experiment whether the desired pH was being obtained. Flour suspensions were made up with 10 gm. of flour in 100 cc. of water or of buffer solution (1 part buffer to 3 parts water) and allowed to extract for 20 min. The liquid was then decanted off and the acidity determined. The results are given in Table XIII.

TABLE XIII

pH OF FLOUR SUSPENSIONS

Sample	Unbuffered	Buffered
Flour No. 1	6.00	4.63
Flour No. 2	5.83	4.65
Flour No. 3	5.93	4.63

These acidities are sufficiently close to pH 4.7 to bring the diastatic activity into the flat portion of the pH activity curve lying on either side of the maximum.

It remained to be determined whether or not sodium citrate had any effect on the diastase independent of the acidity of the solution.

This was done in two ways: by comparison of the activity of a buffered sample with that of one brought to pH 4.7 by the use of lactic acid, and by comparison of samples where the buffer had been used undiluted and where it was used in a 3 to 1 dilution. Any effect of the sodium citrate should be more marked where the buffer is used undiluted. The samples were treated by the usual procedure of digesting for 1 hour, clarifying, making up to 200 cc. and determining the reducing sugars in a 50-cc. aliquot. The results given in Table XIV were corrected for the blank. A sample digested in distilled water is included to show the marked increase in diastatic activity when the acidity is controlled.

TABLE XIV

EFFECT OF SODIUM CITRATE ON DIASTATIC ACTIVITY

Method of controlling acidity	Maltose in 50-cc. aliquot, in mg.
None	31
Lactic acid	54
Citrate buffer (undiluted)	52
Citrate buffer (1 pt. to 3 pts. H_2O)	53

These results show that sodium citrate has no appreciable effect on the diastase. This conclusion is confirmed by results recently obtained by Sherman, Caldwell and Dale (74), working with pancreatic amylase.

In Rumsey's original method, no account is taken of the volume of the flour itself when making the suspension up to volume after digestion. This introduces a slight error. Accordingly the volume occupied by 10 gm. of flour in a number of suspensions was determined. It was found that the average volume was 7 cc. Therefore, the practice has been adopted of allowing for this when diluting the digested suspension. The volume varies slightly from sample to sample so that it is impossible to get an exact volume of liquid but by the addition of an extra 7 cc. the resulting volume is closer to the correct value than it would be if no allowance were made for the volume occupied by the flour.

One of the largest sources of error in the determination of diastatic activity by Rumsey's method has been in the determination of the blank. It was difficult for one analyst or a group of analysts working with different samples of the same flour to check their results. It was, therefore, necessary to find an improved method of determining the blank which would give more reliable results.

Rumsey's directions for the inactivation of the blank are rather ambiguous. After describing the procedure for inactivating the active sample and the preparation of the blank he says "....immediately inhibit diastatic activity by clarifying with the sodium tungstate in the manner just described. The blank determination is then carried out in the same manner except that the addition of 0.4 cc. of concentrated H_2SO_4 is omitted on dilution to volume." In this laboratory sulphuric acid has been used in the clarification of all samples, whether blank or active, as there was no apparent reason for omitting it in the case of the blank. An experiment performed in the course of the present investigation shows that it is fortunate that this procedure was adopted. A sample of diastase-free flour was prepared by the method adopted and 0.1 gm. of taka-diastase added to it. This was treated immediately by Rumsey's procedure, omitting the acid, and allowed to digest for 1 hour at 27°C. At the end of this time 259 mg. of reducing sugar was found. The reducing substances added in the taka-diastase calculated as ma'tose amount to only 80 mg. It is evident that when the acid is omitted inactivation is not complete.

Theoretically, to be a true blank, the extraction of the natural sugar of the flour should be allowed to proceed under the same conditions as the digestion. Experiments were undertaken to discover whether this procedure would be sound. The first step was to ascertain whether the quantity of reducing sugar in the aliquot increased with time of extraction. A series of 10-gram samples of flour was suspended and inactivated in the usual way (using acid). They were allowed to extract with occasional shaking for varying lengths of time before being made up to volume, centrifuged, and sampled for sugar determination. The time of extraction was taken as the time from suspension to sampling. The results are given in Table XV.

TABLE XV

EFFECT OF TIME OF EXTRACTION ON VALUE OF BLANK

Time of extraction, in min.	Reducing sugar as maltose, in mg.
10	33
30	39
60	40
90	42

It can be seen that there is an increase in the blank with increase in the time of extraction. This is particularly noticeable in the first half-hour, and suggests that part of the variability of the Rumsey blank (using the acid) is due to differences in the time elapsing between the making of the suspension and the determination of the sugars. The difference of 7 mg. in the blank as determined after 10 min. and after the usual digestion period of an hour is sufficient to invalidate the results completely when the diastatic activity of flour is low. The total activity in Rumsey units may not equal more than 50 mg. of maltose.

The next step was to determine whether there was any increase in the copper reduced owing to the hydrolysis either of the starch or of the soluble material by the acid used in inactivation. A large sample of diastase-free starch was prepared by the method outlined in the first part of this paper. This was carried through the usual inactivation procedure and allowed to stand. Portions were taken out at intervals and centrifuged, and 50 cc. aliquots were taken for the determination of sugars.

TABLE XVI

HYDROLYSIS OF STARCH FOLLOWING CLARIFICATION

Time of standing in hr.	Copper from 50 cc. aliquot, in mg.
1	0.95
2	0.95
3	1.02

The figures given in Table XVI show, as was to be expected, that the amount of copper reduced is negligible, and that none of the increase reported in Table XV can be attributed to hydrolysis of the starch.

To determine the amount of hydrolysis of the soluble carbo-hydrates a large sample of flour was suspended, inactivated, and centrifuged immediately. The supernatant liquid was allowed to stand, aliquots for the determination of sugars being taken at intervals. The amounts of copper reduced are given in Table XVII.

TABLE XVII

HYDROLYSIS OF SUPERNATANT LIQUID FOLLOWING CLARIFICATION

Time of standing in hr.	Copper from 50 cc. aliquot, in mg.
0.	8.74
0.75	8.61
1.00	8.80
2.00	9.70
26.00	17.67

It will be seen that hydrolysis does proceed but that the effect is negligible during the first hour. We must conclude, therefore, that the increase reported in Table XV is due to the increased extraction of the sugar present in the flour.

Procedure for Determining the Blank in Diastatic Activity Determinations.

The procedure adopted for determining the blank in diastatic activity determinations is as follows: The sample is suspended in distilled water, inactivated according to Rumsey's directions for inactivating the active sample (with acid) and extraction is allowed to go on for 60 min. at 27°C. An aliquot is taken and the reducing sugars are determined immediately. A test of this method was made by determining the blank on four 10-gm. samples of one flour. The results of this test are given in Table XVIII and show much better agreement than was possible using the old procedure. The modifications outlined in this section were used in all the diastatic activity determinations reported in this paper.

TABLE XVIII

QUADRUPLICATE DETERMINATIONS OF BLANK BY METHOD ADOPTED

Sample	Reducing sugars as maltose, in mg.
1	27
2	26
3	25
4	27

Effect of Fineness of Grinding on Resistance of Starch to Diastatic Action.

Through the courtesy of Dr. T. A. Pascoe of the University of Minnesota the writer was able to obtain a very fine series of samples for this study. The flours were from three different wheats, one set of samples (E) being milled in the laboratory experimental mill and the other set (P) in the Minnesota State experimental mill. Thus we have three pairs of flours from the three wheats, differing in fineness of grinding. One of the flours (401P) was further ground for 4, 8 and 20 hours in a ball mill, giving three more degrees of fineness. The figures for starch resistance are given in Table XIX.

TABLE XIX

EFFECT OF FINENESS OF GRINDING ON STARCH RESISTANCE

Sample	Starch Resistance
387E	94
387P	40
392E	78
392P	53
401E	72
401P	37
401P (ground 4 hrs. in ball mill)	30
401P (ground 8 hrs. in ball mill)	28
401P (ground 20 hrs. in ball mill)	24

It will be seen that each of the three flours showed increased susceptibility to enzyme attack as the fineness was increased. Alsberg (3, 4) has pointed out that the increase in diastatic activity on grinding may be due to an increase in the number of damaged grains or to the splitting up of the flour particles which allows the enzyme easier access to the substrate. Ziegenspeck (89) also directed attention to the packing of the granules into lumps being a possible factor in starch resistance. In the present experiment, this factor is probably more important than injury to the granules, since even if there were extensive injury any soluble material would be removed by the washing during the determination, so that any increase in the extractable fraction of the starch owing to grinding would have no influence on the result of the determination. Moreover, a microscopic examination of Flour 401P, ground for twenty hours in the ball mill, showed very few starch grains that would stain with congo red, which they should do if there was any injury to the outer layers (33). This observation agrees with the results obtained by Shollenberger and Coleman (76). They found that the percentage of solids soluble in cold water decreased rather than increased in the finer separates of normal flour. There could not, therefore, have been any appreciable proportion of injured grains.

Effect of Extraction with Ether on the Diastatic Activity and Starch Resistance.

The researches of Taylor and his co-workers (81, 83) have shown that there is a certain amount of fat intimately associated with the starch granule. The concentration of this fat was shown to vary by Aoi (8), who also showed that the extraneous fat associated with starch varied in amount. In view of the fact that lipoids have a marked effect on surface tension and that absorption probably plays an important part in enzyme action (1, 10, 11) it is altogether likely that fats in both forms affect the diastatic action. The extraneous fat may have another effect, for, as Ziegenspeck (89) has pointed out, the permeability for diastase of the layers surrounding the starch grain plays an important role in digestibility.

It has been shown by Taylor and Nelson (81) and by Rask and Phelps (61) that ordinary ether extraction will not remove the lipoids which are combined in the granule. It is, therefore, possible to investigate the two classes of lipoids separately. It was decided for the time being to confine the experiments to the extraneous fat.

The first step was to determine the effect of ether on the diastatic activity and starch resistance without extracting any of the lipoids. This was done by moistening the flour with ether and allowing it to evaporate. The experiment on the effect of this treatment on diastatic activity was carried out with Flour H, but unfortunately samples of this flour were not available for the other experiments reported in this section. Flour A was used for studying the effect of treatment with ether on the starch resistance.

TABLE XX

EFFECT OF TREATMENT WITH ETHER ON DIASTATIC ACTIVITY AND STARCH RESISTANCE

Treatment	Diastatic activity	Starch resistance
None	208	90
1 hour in ether	220	—
24 hours in ether	—	74
48 hours in ether	227	—

It will be seen in Table XX that simple contact with ether lowered the resistance of the starch and increased the value of the sample in Rumsey units. While these changes might be caused by a direct effect of the ether on the enzyme, it is more probable that they are due to an altered distribution of the lipoids. As the ether evaporates it brings the fats to the surface of the mass of flour, and even though the samples were shaken up repeatedly during the evaporation, a change in the location of the fat must have taken place.

In the next experiment, reported in Table XXI, samples of Flours A and B were extracted with ether for 24 hours in Soxhlet's apparatus and diastatic activity and starch resistance were determined on both the extracted and unextracted flour. In weighing out the samples, allowance was made for the amount of fat extracted.

TABLE XXI

EFFECT OF ETHER EXTRACTION ON DIASTATIC ACTIVITY AND STARCH RESISTANCE

Sample	Diastatic activity	Blank	Starch resistance
Flour A	143	40	90
Flour A (extracted)	177	49	70
Flour B	242	61	48
Flour B (extracted)	286	61	35

With both flours the resistance of the starch to diastatic action was lowered and the diastatic activity increased. It has been pointed out that lecithin (36, 84) and certain of the oleates (78) may have an inhibiting effect on diastase. However, it is improbable that this phenomenon affects the results to any marked degree. The increase in diastatic activity in the experiment reported in Table XX must have been due to some other factor, as these substances were not removed. Moreover, the increase in diastatic activity in the second experiment (Table XXI) is not greater than we would expect from the results of the starch resistance determination.

Recently Johnston (34) reported an experiment on the effect of ether extraction on diastatic activity. He found that in every case the extracted flours gave higher values. In discussing his results he says, "As the flour particles were much finer after extraction, it is suggested that the starch in the natural flour may exist in relatively large clumps of granules held together by fatty material. When this fatty material is removed the clumps fall apart, thereby forming a more suitable substrate for the diastase". This suggestion is supported by the results of the starch resistance determinations reported in Table XXI.

Johnson's statement in his summary, that the "reducing sugar content was higher in the ether-extracted flours than in the corresponding natural flours", is misleading, and must be interpreted in the light of two sentences in his discussion: "The suggestion that the removal of fatty material from flour allows the clumps of starch to fall apart also explains the higher reducing-sugar content of the extracted flour as compared with the natural flour. The clumps of starch may occlude reducing sugars which are extracted for determination only when the occluding material falls apart."

Johnson does not describe his method of extracting the reducing sugars, but it is assumed from his method of reporting the results that he accepted the results of the Rumsey blank as a measure of this constituent in the flour. The results given in Table XV show that the blank of the diastatic activity determination, whether determined according to Rumsey's directions or by

the modification outlined in this paper, is not a reliable measure of the reducing sugar content of the flour. This is especially true when Rumsey's directions are followed.

The values of the blanks obtained by the modified method are reported in Table XXI. The most interesting feature of these results is that with Flour A extraction increased the blank while in Flour B the blanks were identical. This can probably be explained by the difference in granulation of the natural flour. Flour A, which was milled in the laboratory mill, has a much coarser granulation than Flour B (a commercial patent). After extraction, the granulation appeared to be very similar, in the A and B flours, and not markedly finer than the original commercial patent. Alsberg and Griffing (3) report that the increase by continued grinding, in the percentage of the flour constituents extracted by cold water, is due principally to the increase in reducing substances extracted. We would expect, therefore, that changes in granulation from any cause would lead to an increase in the extractable reducing sugars. Since there is a marked change in granulation with Flour A, there is a marked change in the blank. With Flour B, although there is a change in the character of the particles on extraction, the fineness is not greatly affected and consequently there is no change in the blank.

Effect of Environment, Variety, and Stage of Maturity at Cutting on Diastatic Activity and Starch Resistance.

A series of flours from wheat grown in connection with another project was available for this study. After cutting, all these samples were carefully protected from weathering. The results are given in Table XXII. The figures for starch resistance were corrected for the protein content of the flour.

TABLE XXII

EFFECT OF PLACE OF GROWTH, VARIETY AND MATURITY OF WHEAT ON DIASTATIC ACTIVITY AND STARCH RESISTANCE

Lab. No.	Place grown	Variety	Stage of maturity at cutting	Diastatic activity	Starch resistance
ERB1	Edmonton	Red Bobs 222	Slightly green	222	51
ERB8	Edmonton	Red Bobs 222	Mature	193	52
EM1	Edmonton	Marquis	Slightly green	208	56
EM8	Edmonton	Marquis	Mature	181	62
SM1	Saskatoon	Marquis	Slightly green	173	54
SM8	Saskatoon	Marquis	Mature	161	51
SG1	Saskatoon	Garnet	Slightly green	189	53
SG8	Saskatoon	Garnet	Mature	219	45

Effect of Environment. This is shown by comparison of EM1 with SM1 and EM8 with SM8. In both cases it will be seen that while the diastatic activity is lower in the Saskatoon samples the starch is more easily attacked. This indicates that the activity of the enzyme is greater in the Edmonton-grown samples.

Effect of Variety. Study of the corresponding 1 and 8 samples of two varieties at each place gives us this comparison. The diastatic activity and starch resistance vary inversely with each other. Thus it appears that the difference in diastatic activity between the two varieties is due principally to differences in the resistance of the starch.

Effect of Stage of Maturity at Time of Cutting. This is shown by a comparison of the "1" samples with the corresponding "8" samples. In the Edmonton series the diastatic activity decreases and the resistance of the starch increases with the delay in cutting. The resistance of the starch is apparently the main factor affecting the result of the Rumsey determination recorded as diastatic activity. With the Saskatoon series the results are quite irregular. In both varieties the starch in the mature sample is more susceptible to enzyme attack. In spite of this, the mature Marquis shows a lower diastatic activity than the corresponding green sample, indicating that there must have been a marked decrease in the activity of the enzyme.

Recently Mangels and Stoa (50) reported an extensive study on the effect of stage of maturity at cutting on the quality of Marquis wheat. They concluded that the diastatic activity showed a tendency to decrease towards maturity. The results from three out of the four pairs of samples studied here are in agreement with this. Bracken and Bailey (15) have studied the effect on wheat of standing in the field after ripening, and report that the diastatic activity shows little change. Tascher and Dungan (80) working with corn have found that the activity of the extracted diastase decreases as the grain approaches maturity.

On account of the small number of samples employed, it is not possible to draw any general conclusion as to the effect of any of the factors on diastatic activity or starch resistance. The data, however, support the findings of Hermano and Rask (31) and Mangels (49) that variations in these properties exist between varieties and between samples of the same variety grown at different places. The results emphasize the necessity of studying the starch resistance as well as the diastatic activity in investigations involving the growing of a number of varieties at different places.

The Effect of Sprouting on Diastatic Activity and Starch Resistance.

A quantity of Red Bobs 222 wheat was divided into four samples, one of which was milled without further treatment and the other three were sprouted for varying lengths of time before being milled. The diastatic activity and starch resistance are given in Table XXIII. The starch resistance figures were corrected for protein content.

TABLE XXIII

EFFECT OF SPROUTING ON DIASTATIC ACTIVITY AND STARCH RESISTANCE

Description of sample	Length of sprouts in inches	Diastatic activity	Starch resistance
Unsprouted	—	146	75
Short sprouts	0–¼	391	63
Medium sprouts	¼–¾	552	69
Long sprouts	¾–1½	834	70

The increase in diastatic activity on sprouting is so well known as to need no comment. The results of the starch resistance determination are peculiar. The starch resistance decreases and then rises again as sprouting progresses. This initial decrease is in agreement with Ling's work (41) with raw and malted barley, but any attempt to explain this behaviour would at present be mere speculation. The increase in the values after sprouting has started is somewhat easier to understand. Whymper (87) noticed that during germination some of the grains were readily attacked and almost disappeared before others showed any marked breaking down. It may be that some at least of the more readily hydrolyzable starch is removed during the period of sprouting and that the resistance of the remaining starch is thereby enhanced.

In any event, the differences in the starch resistance are not great, especially when they are compared with the differences in diastatic activity. There is no doubt that the increase in the values obtained by Rumsey's method is due to an increase in the amylolytic power of the enzyme content.

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STEAM CURING OF PORTLAND CEMENT MORTARS. A NEW CRYSTALLINE SUBSTANCE[1].

(Preliminary Paper)

By T. Thorvaldson[2] and G. R. Shelton[3]

Abstract

The steam-curing of Portland Cement Mortars in saturated steam at 100°, 125°, 150°, 175° and 200° C. was studied both as to variations in the tensile strength of 2-day and 28-day mortar briquets and as to the changes which occurred in the crystalline matter in the cement. The rate of hydration of the cement, as shown by the disappearance of the original crystalline material, increased with the temperature of the saturated steam. Crystals of calcium hydroxide appeared almost at once, but after reaching a maximum decreased again in amount. At the same time, a new crystalline product appeared and increased in quantity as the amount of hydrated lime decreased. Some of the chemical properties of the new crystals are given. The stability of the new crystals when exposed to solutions of sulphates indicates that the great increase in the resistance of Portland cement mortars to alkali action produced by steam-curing is connected with the production of this crystalline material.

Introduction

The soundness of a Portland cement is generally tested by exposure of a specimen of set neat cement to steam at 98 to 100°C. or to boiling water. The cement is considered unsound if a properly prepared specimen cracks, expands above a certain limit, or disintegrates during the treatment. The test may be considered as an attempt to determine the chemical stability of the set cement, and one might therefore expect that it would have some value in the selection of cements which are to be used in structures exposed to destructive chemical agents.

Work done at the U.S. Bureau of Standards (7, 8) indicates that greatly increased strength is conferred on Portland cement mortar and concrete specimens by high-pressure steam curing, provided that the cements are sound in steam at the pressure used. The conclusion reached, however, was that "cement passing the high-pressure steam test does not make more permanent or durable concrete than cement which meets the requirements of the standard specification but fails to pass this test".

Dalton G. Miller (1, 2, 3) has found that the resistance of concrete to alkali water is greatly increased by proper steam curing. A report of a two-year study by Thorvaldson and Vigfusson (6) on the effect of steam treatment of Portland cement mortars on their resistance to the action of solutions of sodium sulphate and magnesium sulphate shows that prolonged curing in saturated steam at 100°C. renders the mortars almost completely resistant to the action

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of solutions of sodium sulphate and increases very materially the resistance of the mortar to the action of solutions of magnesium sulphate. Further studies have shown that immunity to sulphate disintegration is attained much more rapidly in saturated steam at temperatures between 100° and 175°C., than at lower temperatures.

The study reported in this paper was begun with the object of discovering the reason for the increased resistance of cement mortars brought about by steam-curing. A number of commercial Portland cements, which varied greatly in their resistance to sulphate action, were used, as well as a "white" cement made in the laboratory from alumina, white marble, and flint. The method of preparation and the properties of this last named cement have been described by Shelton (4).

Although the commercial Portland cements used were from four different mills the physical tests showed them to be very uniform products in respect to fineness, normal consistency, time of setting and tensile and compressive strength. They were all well above the minimum requirements of the standard specification and passed the test for soundness in steam at 100°C. The "white" cement, to which no retarder was added, had a flash set and short time of setting. It was therefore rather difficult to prepare uniform test pieces from this cement.

Table I gives the chemical analyses of the cements used while Table II gives the results of determinations of their relative resistance to the action of solutions of sodium sulphate:

TABLE I

CHEMICAL ANALYSES*

Cement No.	124	81	132	133	134	"White"
SiO_2	23.82	21.77	20.88	20.15	20.17	24.01
Al_2O_3	5.90	5.99	7.20	7.52	7.31	9.05
Fe_2O_3	1.91	2.52	2.18	2.37	2.81	0.29
CaO	63.58	61.69	61.54	61.94	63.35	66.14
MgO	2.20	3.84	3.61	3.61	3.09	0.36
SO_3	1.74	1.84	1.95	1.60	1.76	trace
Loss on ignition	0.81	2.44	2.66	2.29	1.33	0.30

By D. Wolochow and F. L. P. Steele.

Relative Resistance to Sulphate Action

The relative resistance of these cements to action of sodium sulphate was determined by the expansion method (5). Mortar bars 5/8 x 5/8 x 7½ inches in dimensions, made of one part of cement and ten parts of standard sand by weight, with the amount of water used for a standard 1:3 mix, were cured in water for 28 days and then immersed in 0.15 M. (2.1%) and 0.50 M. (6.7%) solutions of sodium sulphate. The average time, in days, required for linear expansion of 0.1, 0.5 and 1.0% is given in Table II.

TABLE II

RELATIVE RESISTANCE TO SULPHATE ACTION

Cement No.	Time in days				Ratio ("White" cement = 1.0)
	Expansion 0.1%	Expansion 0.5%	Expansion 1.0%	Sum	
124	12.5	30	45	87.5	4.6
81	12	23.5	34.5	70.0	3.7
132	7	10.5	15.5	33.0	1.7
133	7	11.5	14	32.5	1.7
134	7.5	13	15.5	36.0	1.9
"White"	5.0	6.4	7.6	19.0	1.0

The ratios may be considered to represent roughly the relative resistance of these cements to the disintegrating action of solutions of sodium sulphate.

Tensile Strength of Steam-Cured Briquets

The briquets were made of one part of cement to five parts of standard Ottawa sand by weight, with the amount of water prescribed for a 1:3 standard mix. They were left in the damp closet for 48 hrs., then removed from the moulds and immediately exposed to saturated steam. At the end of the period of treatment they were kept in a damp closet for one hour before the tension tests were made. Each value in Tables III and IV represents the average result for at least four test pieces.

TABLE III

1:5 BRIQUETS, 2 DAYS OLD, STEAMED IN AUTOCLAVE AT 150° C.

Time of Steam Treatment	Tension in lbs. per sq. in.					
	Cement No. 124	Cement No. 81	Cement No. 132	Cement No. 133	Cement No. 134	"White" Cement
0	64	67	68	50	69	68
15 min.	26	38	25	6	45	22
30 min.	24	42	26	4	39	29
1 hr.	35	62	33	3	59	42
6 hrs.	95	107	93	0	95	91
12 hrs.	140	149	100	9	148	107
24 hrs.	177	187	195	19	164	180
48 hrs.	198	232	231	34	165	206

TABLE IV

1:5 BRIQUETS, 2 DAYS OLD, STEAMED IN AUTOCLAVE FOR 6 HOURS.

Temperature of Autoclave	Tension in lbs. per sq. in.					
	Cement No. 124	Cement No. 81	Cement No. 132	Cement No. 133	Cement No. 134	"White" Cement
100°C.	58	70	64	42	75	42
125°C.	60	77	79	14	82	58
150°C.	86	107	93	9	97	91
175°C.	140	129	148	—	148	100
200°C.	123	84	102	—	97	59

Similar experiments were made after curing the briquets in water at 21°C. for 28 days. The results are given in Tables V and VI.

TABLE V

1:5 BRIQUETS, 28 DAYS OLD, STEAMED IN AUTOCLAVE AT 150° C.

Time of Steam Treatment	Tension in lbs. per sq. in.				
	Cement No. 124	Cement No. 81	Cement No. 132	Cement No. 133	Cement No. 134
0	182	204	209	184	232
15 min.	94	90	85	98	103
30 min.	113	109	90	90	114
1 hr.	105	110	90	102	139
6 hrs.	117	170	104	101	210
12 hrs.	179	182	136	163	223
24 hrs.	245	238	218	237	265
48 hrs.	256	274	238	205	246

TABLE VI

1:5 BRIQUETS, 28 DAYS OLD, STEAMED IN AUTOCLAVE FOR 6 HOURS.

Temperature of Autoclave	Tension in lbs. per sq. in.				
	Cement No. 124	Cement No. 81	Cement No. 132	Cement No. 133	Cement No. 134
100°C.	146	163	144	129	148
125°C.	104	136	110	77	148
150°C.	116	170	102	101	210
175°C.	206	216	176	190	223
200°C.	165	158	167	145	195
225°C.	100			84	

Discussion of the Tension Tests

While cement No. 133 passes the steam test at 100° C. the two-day 1:5 mortar briquets of this cement fail in steam at 125°C. or higher temperatures. After storing the briquets in water for 28 days, their behaviour in steam under pressure does not differ materially from that of briquets made of the other cements.

Considering test pieces made from the cements which pass the steam test at 150°C., one finds (Tables III and V.) that these decrease in tensile strength very rapidly when exposed to saturated steam at 150°C., the point of minimum strength being reached in less than 30 min. Further steaming then produces an increase in strength, the usual 28-day tension being attained with the two-day briquets in about 48 hours.

From Tables IV and VI it is seen that the rate of increase in strength of the briquets in saturated steam increases with rising temperature up to 175 C., while exposure for 6 hours at a temperature of 200°C. does not, on the average, produce much higher strength than at 150°C., and steaming at 225°C. produces very marked decreases in strength.

There is no apparent relation between the sulphate resistance of the cement as given in Table II and the effect of saturated steam on the tensile strength of a 1:5 mortar made from the cement as given in Tables III, IV, V and VI.

Optical Examination of Steam-Cured Briquets

After the briquets had been broken, the hydrated cement was separated from the sand and was subjected to a careful optical examination by means of a petrographical microscope. Three types of change were observed:

1. Unhydrated Cement:

In all cases the amount of unhydrated material decreased and the amount of gel increased with the time of steaming and also with increased temperature of the autoclave.

2. Hexagonal Crystals of Hydrated Lime:

Hexagonal plates of hydrated lime appeared almost at once on steaming and increased in amount with the duration of steam treatment and with the temperature of the autoclave to a maximum, beyond which the amount of these crystals of hydrated lime again decreased. In the case of the two-day briquets steamed at 150 C., the amount of the hexagonal crystals of lime usually began to decrease at the end of six hours and had completely disappeared at the conclusion of the 12-hour period. In the case of the 28-day briquets, these crystals persisted longer, reaching the maximum in from 12 to 24 hours and persisting in small amounts after the 48-hour period. In the series of steam treatments for six-hour periods at different temperatures the quantity of the hexagonal crystals of hydrated lime increased with the temperature of the saturated steam up to 150 or 175 C., then decreased, but some of the crystals were generally present even after a six-hour period at 200 C.

3. A New Crystalline Substance:

After steaming the 2-day briquets for six hours at 150°C. the formation of new crystals was observed, and these increased in number and size on further treatment at this temperature. These were thin elongated plates ordinarily with square ends but sometimes showing bevelled edges. It may be significant that these crystals were usually observed first at the point where the quantity of hexagonal crystals of hydrated lime began to decrease and that the latter gradually disappeared as the amount of the former continued to increase. In the briquets made from the "white" cement, these crystals were not found until after steaming for 24 hours at 150°C., a greater quantity being present after 48 hours.

In the 28-day briquets the new crystals appeared first after 12 to 24 hours of steam treatment at 150°C., and continued to increase in amount on further treatment at this temperature.

When the two-day briquets were exposed for six-hour periods to saturated steam at different temperatures the new crystals appeared at the following temperatures: In cement No. 133, at 100°; No. 132, at 125°; Nos. 81 and 124, at 150°; and No. 134, at 175°C. The crystals continued to increase in amount up to and including 175°C., but were in general not as abundant after six hours at 200 as after 48 hours at 150 C. In the case of the 28-day briquets, the crystals did not usually appear on steaming for six hours at temperatures below 175°C. Prolonged exposure to steam at 175°C. gave the largest amount of the crystals. This, it has been found, is also the temperature producing the most rapid increase in tensile strength of the mortar and the greatest resistance to the action of sulphates.

Optical Properties of the New Crystals

The new crystals are anisotropic with parallel extinction, and are orthorhombic. The indices of refraction are:
$\alpha_{Na} = 1.614 \pm 0.002$, $\beta_{Na} = 1.620 \pm 0.002$ and $\gamma_{Na} = 1.633 \pm 0.002$. Maximum interference colour, crystals on edge, is yellow of the first order. When lying flat, the colour of the crystals is faintly gray and the measured birefringence is 0.006. The character is positive, though an optical figure was obtained with difficulty. The optical angle, $2V = 68°$, (calculated from indices of refraction). Elongation is positive. Twinning is common. Crosses and crystals at right angles are frequently observed and radiating groups of crystals are sometimes found. Figs. 1, 2 and 3 represent photomicrographs of the crystals.

Chemical Properties of the New Crystalline Substance

The new crystals are decomposed by very dilute acids but are stable in solutions of sodium hydroxide. They are not attacked by solutions of sodium or calcium sulphate, but decompose slowly in solutions of magnesium sulphate and are in this respect similar in stability to steam-treated cement mortars.

The fact that the hexagonal crystals of hydrated lime disappear as the new crystals are formed suggests that the latter might be another crystalline modification of hydrated lime. This is unlikely, for when the crystals are decomposed by dilute hydrochloric acid, an insoluble skeleton of the crystals remains. When sulphuric acid is used a large amount of gypsum is formed showing that the crystals are rich in lime.

The crystals are very stable when exposed to heat. Not only were they dried in air at 135°C. for days without any change being noted in them but they were heated at 400°C. for 24 hours before any roughening of the crystal edges was noted. On prolonged heating at 650°C. some of the crystals became very dark but between crossed Nicols appeared bright in some places, showing that their entire crystalline structure had not been destroyed.

Acknowledgment

The authors wish to acknowledge their indebtedness to Mr. V. A. Vigfusson for making the photomicrographs of the crystals.

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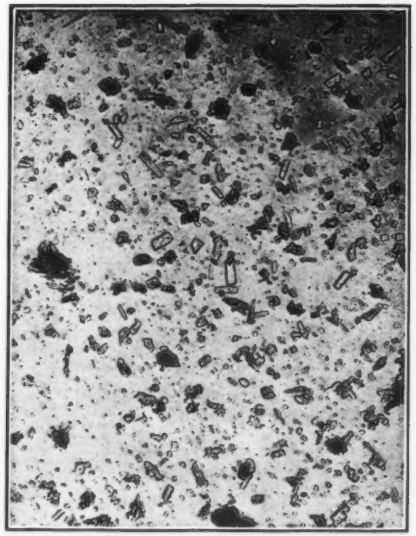

FIG. 1. *Crystals in steam-treated Portland cement mortar. × 100.*

FIG. 2. *Crystals in steam-treated Portland cement mortar. × 360.*

FIG. 3. *Same as Fig. 2 except between crossed Nicols.*

PLATE I

A NEW METHOD FOR THE RAPID ESTIMATION OF MOISTURE IN WHEAT[1]

By E. F. Burton[2] and Arnold Pitt[3]

Abstract

A rapid method of estimating the moisture in a sample of wheat is described. This method depends on the effect produced in a specially arranged radio circuit in which an alternating current of high frequency is generated. When a container holding some of the wheat under examination is introduced into the rapidly alternating electric field, a change occurs in the strength of the current which may be measured by an ammeter in the circuit and which may be immediately interpreted as a measure of the moisture content of the wheat.

Introduction

During the years 1927-1928 the authors (1) conducted, with a radio circuit, a series of experiments designed to measure the dielectric constant of various liquid and solid media. The apparatus was found to be extremely sensitive and to be capable, in particular, of quickly revealing minute differences in the value of electrical properties of ordinary water of varying degrees of purity. It appeared that these experiments might find practical applications in some of the industries, especially where drying processes were involved. Since the measurement of the relative volume of water in the grain is an important factor in the grading of wheat, the procedure suggested itself as an improvement on existing methods both in respect to rapidity and accuracy. The present paper comprises an outline of several well-known means of estimating moisture in wheat and gives the results of a comparison between one of them and the new electrical "Burton-Pitt" method.

Commercial Methods of Moisture Determination in Wheat

The determination of the percentage of moisture is of paramount importance in the grading of wheat and it is necessary that such a determination should be made as quickly as possible. All present commercial methods depend on heating the wheat, either in an oven or in oil, so as to drive off the moisture without causing a chemical change. The percentage moisture is estimated either by the loss in weight of the sample in drying, or by measuring the volume of the condensed water from a given weight of wheat. It is a very difficult matter to drive all the moisture from any kind of grain; something in the colloid structure of the grain tends to retain the last traces of water with great tenacity. Three of the methods which have been devised will be described.

[1] *Manuscript received May 22, 1929.*
Contribution from the laboratories of the University of Toronto, Toronto, Canada, with financial assistance from the National Research Council of Canada, and with the co-operation of Dr. R. Newton, Dr. F. J. Birchard and the staff of the Research Laboratory of the Board of Grain Commissioners at Winnipeg.
[2] *Professor of Physics, University of Toronto.*
[3] *Research Assistant, Department of Physics, University of Toronto.*

Slow Heating in Oven

According to the Association of Official Agricultural Chemists, the grain should be heated in a current of dry hydrogen or in a vacuum at the temperature of boiling water until the weight becomes constant; the time usually adopted is five hours although there is no doubt that the time required to drive off all moisture is really much longer. The American Association of Cereal Chemists has replaced the words "temperature of boiling water" by the phrase "98° to 100° C.", as it is found that heating even to 100° C. for any considerable time brings about a darkening of the sample, which indicates the evolution of decomposition products. The Associate Committee on Grain Research of the National Research Council of Canada has adopted as the most accurate method the procedure of heating the grain in a vacuum oven at 98°C. for 48 hours. In all of these modifications of this method the object is to heat the wheat until it is "bone-dry". In spite of the great length of time required, this method remains as the ultimate standard for moisture determinations.

Rapid Heating in an Air or Vacuum Oven

In this method the time of heating is reduced by maintaining the temperature of the oven much higher than the boiling point of water. About 10 grams of wheat is ground in a special mill, weighed and placed in the oven at 130°C. for one hour. On removal from the oven it is cooled in a desiccator and again weighed.

This method is really only empirical because not all of the moisture is driven out of the grain by heating it for one hour. The portion of the absorbed moisture which remains in the wheat, in spite of the heating, is approximately balanced by water produced by chemical breakdown in the wheat (due to the high temperature) and driven off by the heating process. Hence it has been found that this method of rapid heating gives approximately the same results as the slower method previously described. It is, however, criticised by many workers because minor variations in the temperature of the oven, time of heating, position of sample in the oven and other factors will lead to erroneous results.

The Brown-Duvel Method

The method most widely used is the "Brown-Duvel" (2, 3, 4, 5, 6). It consists in boiling a known weight of wheat in oil of high flash point, condensing the water driven off, and measuring its volume. One hundred grams of wheat is placed in a flask with 150 grams of oil and the whole is heated at such a rate that in twenty minutes it is brought to a temperature of 180°C. It has been found by comparison with the slow-heating method that if a longer time is taken to reach this temperature the results are too low, and if a shorter time, too high. When the temperature of 180° C. is reached the heat is cut off and the whole flask left closed until it has cooled to 160° C. In carrying

out the experiment the thermometer must be adjusted so that four-fifths of the mercury bulb is immersed in the grain and oil; this adjustment is one which must be made with extreme care.

It will be seen that this method is also largely empirical as any variation in the rate of heating, the time of heating, the maximum temperature, the position of the thermometer, etc., will lead to erroneous results—either too much water or too little will be evaporated.

The Burton-Pitt Method

This method depends on the effect produced in a specially arranged radio tube circuit, in which an alternating current of high frequency is supplied by the tube. When a foreign body is introduced into this rapidly alternating electrical field a change occurs in the value of the current flowing—a change which can be measured by an ammeter in the circuit.

Fig. 1 represents the arrangement of an ordinary oscillating circuit. The essence of the discovery connected with this apparatus is that for certain critical values of the inductance and capacity of the circuit, the strength of the current as shown by the ammeter, M, varies greatly when foreign materials, by means of the tube T, are inserted in the core of the coils, L_1 and L_2. The purpose of the experiments outlined below was to determine, in the Dominion Grain Research laboratory at Winnipeg, the nature of the relation existing between the strength of current and the moisture content of wheat samples placed in the tube T. This was done by comparing the ammeter readings with the moisture content as determined by the standard methods already described.

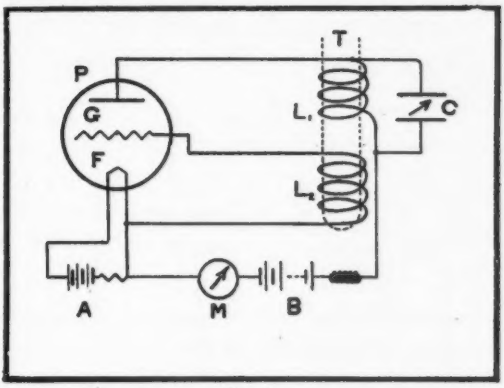

FIG. 1

Outline of Experimental Tests

The samples of different varieties and grades of wheat were supplied by the Research Laboratory of the Board of Grain Commissioners. These samples were of the following varieties: Axminster, Ontario, Marquis and Durum. The grades ranged from a high-grade Marquis to a badly frosted, undeveloped, feed wheat (Table I). As the samples were all quite dry from standing during the winter in the laboratory, moisture was introduced artificially by tempering the original dry samples. The following methods were used in moisture determinations:

(1) Burton-Pitt,
(2) Brown-Duvel,
(3) Air oven,
(4) Vacuum oven.

First Series of Experiments

For purposes of comparison, the final tests of samples, chosen jointly by Dr. Birchard, of the Dominion Grain Research Laboratory, and Mr. Pitt, are given in Table I. The former selects as his most reliable determinations those made by the Brown-Duvel method.

In the case of the Burton-Pitt apparatus, four readings were taken with each sample: two with a small tube—one with the grain loosely packed and one tamped—and two similar readings with a large tube. It had been found in previous work that the most consistent results were given by the larger tube, either with the wheat uniformly poured into the tube or with the wheat packed by tamping for a few seconds. The Burton-Pitt deflections given in Tables I and II are for the larger tube, with the wheat packed by tamping.

In all the tests by all the methods it was revealed that when the grain was tempered it was necessary to allow it to stand for at least twenty-four hours before uniform and consistent results are obtained.

In Table I there are recorded the various readings on the original samples of dry grain and on moistened samples which had been tempered for several hours.

TABLE I

SUMMARY OF FIRST SERIES OF EXPERIMENTS

Symbol	Variety	Wt. per bushel in pounds	Burton-Pitt deflection	Brown-Duvel laboratory	Brown-Duvel Inspection Office	Air oven	Vacuum oven
A_1*	Axminster	62.25	47	7.2		7.27	7.27
O_1	Ontario	59.5	68	14.2		14.81	14.75
H_1	Marquis	64.5	55	10.2		10.00	10.13
D_1	Durum	61.0	55	10.7		10.52	10.47
T_1	Marquis	56.5	47	8.7		8.56	8.51
M_1	Mixture (T & H)	60.5	51	9.6		9.24	9.26
N_1	No. 2	65.0	82	15.6	15.7	15.63	15.56
K_1	No. 5	62.0	80	15.7	15.6	15.64	15.58
Q_1	No. 6	61.0	84	15.8	15.8	15.70	15.67
P_1	Feed Wheat	56.5	74	15.2	15.2	15.13	15.05
A_2			65	13.6	13.5	14.01	13.39
O_2			85	15.8	15.7	16.60	16.02
H_2			66	13.8	13.8	14.04	13.78
D_2			65	13.4	13.4	13.26	13.61
T_2			63	13.6	13.6	13.81	13.63
M_2			63	13.5		13.70	13.53
A_3			86	16.9			
O_3			99	18.1			
H_3			92	16.9			
D_3			87	16.8			
T_3			87	16.8			
M_3			92	16.9			
N_2			105	20.1			
K_2			107	20.0			
Q_2			108	20.2			
P_2			109	20.1			

*NOTE: *A—Axminster, rare variety; O—Ontario, low protein; H—Marquis, heavy weight; D—Durum, macaroni wheat; T—Marquis, thin kernels, low weight; M—Mixture of T and H; N—No. 2; K—No. 5; Q—No. 6; P—Feed Wheat, frosted; A_1 to M_1—Untempered; N_1 to P_2—Tempered 72 hours.*

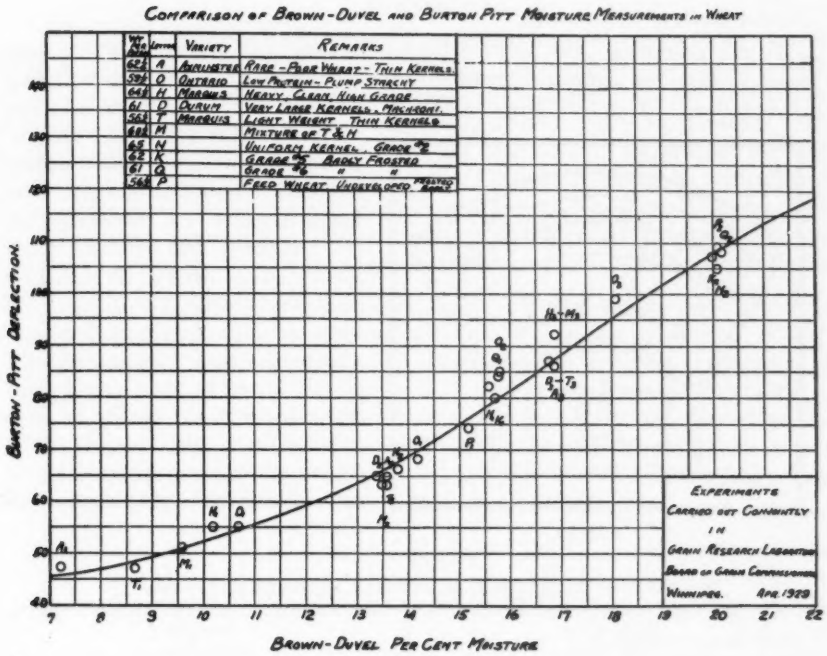

FIG. 2

In Fig. 2 the Burton-Pitt deflections have been plotted against the Brown-Duvel moisture percentages from Table I. It will be observed that the points lie along a portion of a curve which is similar to the theoretical characteristic curve (Fig. 3) pertaining to the electrical circuit. The range of moisture content of commercial interest is approximately that from 12 to 22%. It will be seen that within this range a linear relation exists between the Burton-Pitt readings and the moisture percentages as determined by the Brown-Duvel method. This is of some importance in the practical determination of moisture by the Burton-Pitt method since it facilitates the calibration of the ammeter in such a manner that direct moisture-content readings may be made, as indicated in Fig. 4.

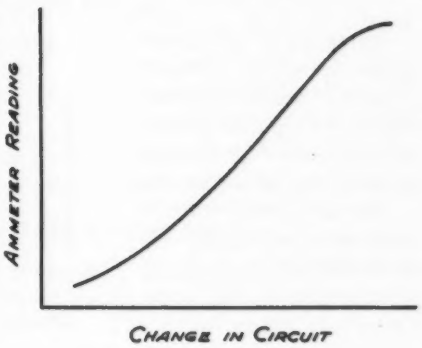

FIG. 3

Second Series of Experiments

In the preceding tests many different varieties and grades of wheat were used; in fact, the samples represented most of the types of wheat encountered in practice. A second series of similar readings was taken with only one variety (No. 3 Northern) tempered so as to give various moisture contents. The moist samples were again prepared by tempering but the tempered samples had stood for 4½ days before the moisture tests were made. As before, the most reliable determination was selected in each case, namely, the Brown-Duvel by the Grain Laboratory and samples packed in large experimental tubes by Mr. Pitt. These results are given in Table II and illustrated graphically by Fig. 5.

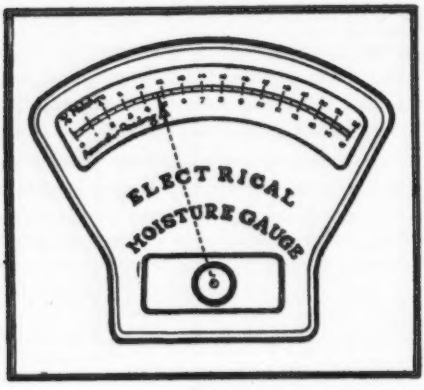

FIG. 4

TABLE II

MOISTURE IN NO. 3 NORTHERN WHEAT

Burton-Pitt deflection	Brown-Duvel percentage
56	11.7
61	12.8
66	13.6
71	14.3
78	15.5
87	16.4
94	17.7
104	18.3
105	19.7

Inspection of the graph again shows the existence of a linear relation between the current in the circuit and the actual percentage moisture except in the case of the eighth reading which apparently reveals an experimental error.

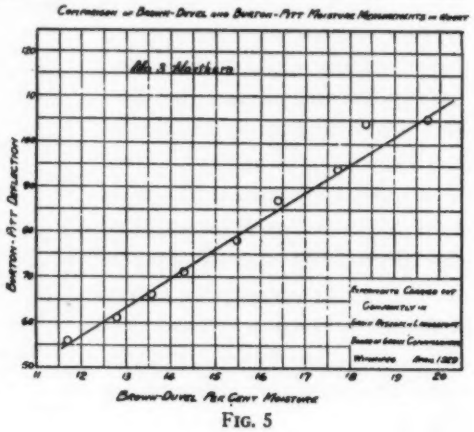

FIG. 5

Discussion

The results given in Tables I and II represent summaries of a large number of experiments. The authors are confident that the above-mentioned simple relation between moisture content and current strength holds for all ordinary grades and varieties of wheat. For the most accurate work, however, it is obviously necessary to standardize the Burton-Pitt apparatus for the particular variety of wheat which is being tested. It should be remembered that the Brown-Duvel method and all the other rapid methods of determining moisture are themselves only approximate. That a departure from the normal procedure may result in serious errors is shown by the following experiments.

Two samples of wheat of approximately the same moisture content, according to the regular Brown-Duvel procedure, but of different grade and variety, were heated as shown in Table III. It will be observed that the amount of moisture evaporated varied greatly with the temperature and the rate of heating and that the two varieties responded differently to the same abnormal treatment. The necessity for careful specification of the conditions of the Brown-Duvel or any other rapid drying method is clear. It is equally clear that the Burton-Pitt method is not similarly restricted.

TABLE III

EFFECT OF ABNORMAL CONDITIONS ON BROWN-DUVEL READINGS

Time p.m.	Temperature in degrees Centigrade	Percentage of Water	
		Marquis	Durum
	Normal Brown-Duvel Readings	15.4	15.3
2.20	Heating Started		
2.35	125°		
2.40	133°	9.3	8.6
2.50	133°	11.0	10.1
	(Water coming off very slowly)		
3.00	(Raised to 148°)		
3.15	148°	13.3	12.9
3.23	(Raised to 175° – 180°)		
	(Water dropped rapidly when 175° was reached)		
3.37	180°	15.6	15.2
3.40	(Raised to 200°)		
4.00	200°	17.7	16.8
4.15	200°	18.6	17.6
4.30	200°	19.0	18.1
5.00	207°	19.9	18.8
5.30	215°	21.6	20.6
	(Heat turned off)		

ADVANTAGES OF THE BURTON-PITT METHOD

1. *The sample of wheat need not be weighed.* A standard test tube is filled with wheat and then inserted in the apparatus, which is so arranged that the tube fits into the proper place in the coils. The actual weight of the sample is not required either before or after the observation.

2. *The reading on the ammeter is practically instantaneous.* The needle of the ammeter at once moves to a definite position. The scale can be calibrated directly in percentage of moisture.

3. *The apparatus is strong and portable.* The particular apparatus used in Winnipeg was originally set up in Toronto, shipped by express to Winnipeg, moved about Winnipeg and set up in three different laboratories, expressed back to Toronto and again set up without any repairs or readjustment of the radio tube or any part of the electrical equipment.

4. *The apparatus requires no artificial heating and its readings are independent of the temperature and humidity of the room in which it is used.* There are no delicate adjustments of temperature necessary. The temperature of the wheat, however, must not be below the freezing point of water.

5. *The accessories are replaceable and relatively inexpensive.*

Battery A—a six-volt automobile battery.

Battery B—a set of dry cells (135 volts).

Radio Tube—an ordinary inexpensive tube.

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THE INHERITANCE OF REACTION TO BLACK STEM RUST OF WHEAT IN A DICOCCUM × VULGARE CROSS[1]

By J. B. Harrington[2] and W. K. Smith[3]

Abstract

A genetical study of resistance of wheat to black stem rust, and a plant breeding attack on the rust problem are described. A large F_2 population of the cross Vernal (*T. dicoccum*) × Marquis (*T. vulgare*) was grown under severe natural epidemic conditions in the field and hundreds of F_3 progenies were exposed in the seedling stage, under controlled conditions, to pure physiologic forms of rust. In the field Vernal is highly resistant and Marquis susceptible to most forms of stem rust. Resistance in the field proved incompletely dominant and appeared to be governed by a single genetic factor. Marquis and Vernal were found to differ by one main genetic factor, R_b, for seedling reaction to form 21. This factor R_b, carried by Vernal, also governs seedling resistance to forms 17, 29 and 36 and appears to be responsible for the slight seedling resistance of Vernal to form 27. There was some evidence that the factor R_b is the same factor that controls the resistance of the F_2 plants to the forms of rust in the field (forms 17, 21, 29 and 36 were known to be present.) A different factor R_a causes the resistance of Marquis seedlings to form 27. Vernal resistance was not found to be associated closely with the seed shape of that variety nor with its adherence of glumes to the seed.

Introduction

During the past twelve years the breeding of wheat for desirable combinations of stem rust resistance and other characters has been carried on energetically. The problem has been found to be highly involved. There are over forty different physiologic forms of *Puccinia graminis tritici*, and the necessity of using inter-specific crosses between wheats of different chromosome numbers further complicates the situation.

Review of Literature

Early work, including the discovery and isolation of different physiologic forms of wheat stem rust, and numerous genetical studies, in which reaction to stem rust was found to be inherited in Mendelian manner, has frequently been reviewed and need not be mentioned here (1, 10, 16).

It is well recognized that serious difficulties must be overcome in the breeding of desirable resistant wheat. One of these is the increasing number of physiologic forms of black stem rust. Since 1922, when Stakman and Levine (35) described 37 of these forms, several new ones have been reported in Canada by Newton, Johnson and Brown (24, 26, 27). A plausible explanation of the origin of these apparently new forms lies in the recent discovery of Craigie (5, 6, 7) on the function of the pycnospores. His results indicate the possibility

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Contribution from the laboratories of the University of Saskatchewan, Saskatoon, Saskatchewan, Canada, with financial assistance from the National Research Council of Canada. This study forms part of a co-operative attack on the problem of cereal rust in Canada, carried on jointly by the National Research Council, the Federal Department of Agriculture and the Universities of Alberta, Manitoba and Saskatchewan.
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of new physiologic forms of wheat rust originating through hybridization between different forms on the barberry leaf. New forms may also arise through mutation. Newton and Johnson (25) found in form 9 a mutant with orange-coloured pustules. They also found a grayish-brown mutant in form 36, obtained from aecia on barberries. Both mutants remained constant in pure culture and reacted on the wheat "differentials" in the same way as their parent forms. A wheat variety to be thoroughly desirable should therefore possess resistance to all of the known forms of stem rust and should have the genetic make-up that would probably yield resistance to new forms that may develop.

Another difficulty is that of chromosomal incompatibility between species of different chromosome numbers. Crosses between *vulgare* wheats with 21 chromosome pairs and varieties of *dicoccum* and *durum*, both of which have 14 chromosome pairs, have been found necessary in the building up of desirable new hybrid combinations of characters. In such crosses chromosomal incompatibilities usually result in high sterility in F_1 and relatively few F_2 plants of intermediate character. Sax (33) studied 38 F_3 segregates of a *T. vulgare* × *T. durum* cross and found a very high degree of association between chromosome number and morphological and physiological characters including stem rust resistance. He concluded that it might be impossible to combine in a homozygous condition the desirable characters of wheats containing 14 and 21 chromosomes.

In a somewhat similar study Thompson (36) obtained a few rust resistant *vulgare*-like segregates, although not one was as resistant as the *durum* parent.

Hayes and others (15, 16) have made extensive studies on the cross Iumillo (*T. durum*) × Marquis (*T. vulgare*) and not only found it possible to obtain stable combinations in which both *durum* and *vulgare* characters were present, but succeeded in producing a highly desirable new hybrid variety (Marquillo), possessing a large part of the rust resistance of Iumillo and nearly all of the superior qualities of Marquis.

During recent years the results of several studies of the inheritance of resistance to stem rust in *durum* × *vulgare* hybrids have been published. No clear indications of simple Mendelian ratios were found and the number of factors involved could not be determined definitely.

Thompson and Hollingshead (37) have reported the results of a genetical study of the cross *T. dicoccum* × *T. vulgare*. They observed a striking preponderance of *dicoccum* types in the segregates. It was suggested that the results obtained might have been due largely to even greater chromosome incompatibilities than in the cross *T. durum* × *T. vulgare* which they had studied.

There has been comparatively little investigation into the inheritance of resistance to stem rust in crosses between the species *dicoccum* and *vulgare*. Hayes, Parker and Kurt (15) studied a small group of 73 hybrid lines from crosses between White Spring emmer and Marquis. In F_2 no resistant *vulgare*-like plant was found, but in F_3 several plants were obtained which not only were rust resistant but also resembled common wheat.

The high resistance of Khapli (*T. dicoccum*) to all known forms of stem rust would be particularly desirable in a bread wheat, but tremendous difficulties, such as lack of vitality and fertility in hybrids, have been encountered in attempts which have been made to combine it with the necessary bread wheat qualities. Harrington (11) in 1926 found that the *dicoccum* varieties, Vernal and Early emmer, crossed readily with Marquis. Vernal has high resistance to a large number of the identified forms of stem rust. The suitability of a cross between Vernal and Marquis for a study of the inheritance of rust reaction and the possibility of the solution of the rust problem by such a cross suggested the present study.

Materials Used

Vernal (*T. dicoccum*) C.I.3686, was used under the name White Spring emmer C.I.3686 by Stakman and Levine (35) in their key for the identification of physiologic forms of wheat stem rust. This variety has been grown in the cereal breeding nursery at Saskatoon for several years and is uniform in morphological type. It has high resistance to 32 of the 37 physiologic forms of rust identified by Stakman and Levine (35) and to three of the forms recently isolated by Newton and Brown (26). It is susceptible or moderately susceptible to the other seven forms according to seedling tests made in the greenhouse, but in the field (Fig. 1, Plate II) it rarely shows an appreciable amount of infection (21). Since resistance under field conditions is the only resistance of importance to the farmer, it is evident that the variety Vernal is highly satisfactory in this respect.

Marquis (*T. vulgare*) Sask. 7 is a strain of Marquis obtained indirectly from the Central Experimental Farm, Ottawa, about fifteen years ago. This variety, while outstanding in yield, baking quality and other respects, is susceptible (Fig. 1, Plate II) to a large number of the rust forms. The Sask. 7 strain of Marquis has been grown continuously at Saskatoon for many years, and at the time the crosses were made was one of the most extensively grown strains in Saskatchewan.

Pure cultures of several physiologic forms of *P. graminis tritici* were employed for the seedling tests made in the greenhouse. Forms 17, 21, 29 and 36 were obtained from the Dominion Rust Laboratory, Winnipeg, whose co-operation in this regard has been much appreciated. Forms 17, 21 and 36 were used because of their importance in Western Canada. Form 17 was the predominating form in Western Canada during the years 1919 to 1921 and was abundant in 1922 and 1923. During the years 1925 and 1926 forms 21 and 36 were much more prevalent than any others (24). In 1927, forms 17, 21 and 36 occurred extensively in Canada and form 29 was fairly prevalent.

The writers are indebted to Dr. M. N. Levine of the University of Minnesota for a culture of form 27 which he supplied. This form was desired because, unlike form 21, it attacks Vernal in the seedling stage but not Marquis.

Nursery Methods

The parent varieties, F_1 and F_2, were grown in five-foot rows, the plants being several inches apart in the rows. During the season of 1927 observations were made on rust infection, plant height and some spike and seed characters of the F_2 hybrids.

Rust Infection

In 1925 and 1926 there was not much rust in the nursery, but in 1927, owing to exceedingly favorable environmental conditions, the rust epidemic was very severe. Rust forms 17, 21, 29, 36 and C[1] were found to have participated in this epidemic and possibly others were also present. The reaction of the plants to rust in the nursery was recorded just prior to full maturity. Two observations were made on each plant, one being the percentage infection of the stem and the other the character of the reaction. The latter was made on the basis of the vigor, width and size of the rust pustules by means of five expressions, viz: resistant (1), medium resistant (2), medium (3), medium susceptible (4), and susceptible (5). For example, the pustules on a plant classed as resistant were small and narrow and had broken through the epidermis only slightly or else not at all.

Height of Plant

Marquis and Vernal do not differ appreciably in height at Saskatoon, but in the hybrid progeny of a cross between these varieties there is a wide range of height. This is to be expected where, in addition to the ordinary genetic factors governing height, chromosomal irregularities violently upset the course of Mendelian inheritance. Height of plant was studied in order to determine the possible relation of that character to other characters studied.

Adherence of Glumes to Seeds

In Vernal the lemma and palea tightly clasp the seed; in Marquis they can be pulled apart fairly easily. This characteristic is of considerable importance from the economic standpoint. The F_2 hybrids were grouped into five classes—the parental types and three intermediate classes—on the basis of the percentage of seeds that were free from the chaff after threshing.

Seed Shape

Vernal has a long narrow seed tapering at both ends while the kernel of Marquis is short, broad and blunt. Five gradations were established as in the case of the preceding characteristic.

Fertility

The percentage of seed-setting on resistant segregates of the *vulgare* type is of some interest. In the parent varieties the seed-setting of florets on the spikelets at the tip and base of the spike, as well as of the tertiary and later developed florets of any spikelet is readily affected by environmental conditions.

[1]*The temporary designation of one of the new forms isolated at the Rust Laboratory, Winnipeg.*

FIG. 1. *Representative spike, culm and seed material of the cross Vernal × Marquis grown in the field in 1927 under the conditions of a severe natural epidemic of black stem rust. A—Marquis, susceptible. B—Vernal × Marquis F_1, resistant. C—Vernal, highly resistant. For each variety the section of culm at the left is from the neck (peduncle) and that at the right includes the top node. The seed of Vernal and of Vernal × Marquis F_1 is not free-threshing. Those shown as naked seeds were removed from the investing chaff.*

(NOTE: *The photograph was 'touched up' to make the rust reactions look as nearly as possible as they did in the field at the time the material was gathered.*)

FIG. 2. *The Greenhouse Reaction of Seedlings of Vernal, Marquis and Vernal × Marquis F_1, to two Forms of Stem Rust to which Vernal and Marquis React Reciprocally.*

Top Row:—Reaction to physiologic form 27 showing the incomplete dominance of resistance. A—Marquis, resistant. B—Vernal × Marquis F_1, moderately resistant. C—Vernal, moderately susceptible.

Bottom Row:—Reactions to physiologic form 21, showing the incomplete dominance of resistance. D—Marquis, susceptible. E—Vernal × Marquis F_1, resistant. F—Vernal, highly resistant.

(NOTE:—*The photograph was 'touched up' to make the rust reactions look as nearly as possible as they did in the greenhouse at the time the leaves were removed from the seedlings.*)

To determine as accurately as possible the number of florets which failed to set seed for reasons other than environmental, the spikelets on the upper and lower sixths of each spike were disregarded. On the remaining two-thirds of the spike, only the fertility of the primary and secondary florets of each spikelet was considered.

Greenhouse Methods

The methods employed in this study were essentially the same as have been used in somewhat similar investigations during recent years (2, 10, 23). Greenhouse tests of the reactions of the parent varieties, and the F_2 and F_3 seedlings to pure cultures of rust forms were made during the winter. The seedlings were grown in sterilized soil in five-inch pots, approximately 20 seeds being sown in each pot. When the seedlings were about 2½ inches high they were inoculated with a culture of stem rust by the brush method, in which pots of plants heavily infected with rust are inverted and brushed lightly to and fro over the seedling leaves which have been previously wetted by a fine spray of water. This method would not be feasible where a large number of forms were to be kept for a long period.

After inoculation the pots of seedlings were placed in moist chambers for 48 hours. A temperature the same as, or lower than, that of the greenhouse was maintained within the incubators by the addition of pieces of ice. Peltier (26, 27) found that different physiologic forms of stem rust had different optimum temperatures for initial infection but that temperatures from 20° C. to 25°C. suited forms which he had under study. Without the use of ice or of a continuous circulation of cool water through the incubation chambers the temperature within these chambers tends to rise too high, particularly if they are not well shaded from the sun.

After incubation the pots were placed on benches which, in the short days of winter, were illuminated during the hours of darkness by 100-watt lamps, hung two feet above the bench and equipped with deep bowl porcelain reflectors. The use of artificial light was found by Harrington (11) to promote the growth of wheat plants during the winter. It was observed by the writers in the winter of 1925-1926 that artificial light aided the development of rust on wheat seedlings, probably because it promoted vigorous growth of the young plants. Peltier (29), in an experiment on the use of artificial light in work with rust, found "a decided difference in the development of rust in the different light compartments". He states that "the most rapid development occurred in the compartments having the greatest intensities of light".

Double cotton partitions were used to separate material inoculated with different forms. Infection of the individual seedlings was recorded 15 to 20 days after inoculation, depending upon the prevailing weather conditions. For example, extremely cold weather caused darkening of the greenhouse due to the formation of a thick sheet of ice on the glass roof, with consequent retardation of rust development on the seedlings.

Record of Rust Reaction

The observations on infection of seedlings in the greenhouse were based on the Stakman and Levine criteria (35) modified to suit the genetical analysis of hybrid material where the seedlings show all gradations of reaction between the parental varieties.

Purity of Forms

The purity of the rust forms used in the greenhouse throughout this study was determined weekly by inoculation of sets of 12 "differential" varieties of wheat (35).

Experimental Results

Vernal was crossed with Marquis in 1925. Marquis was used as the male parent on account of the abundance of good pollen it produces. The average seed-setting in the Vernal florets pollinated with Marquis was 60%, or an average of 10 F_1 seed on each mother-plant spike. The F_1 plants were grown in 1926, and from them 2,950 F_2 seeds were obtained.

TABLE I

REACTION OF VERNAL × MARQUIS F_1 AND F_2 SEEDLINGS TO RUST FORM 21 IN THE GREENHOUSE IN 1927-1928

Parent variety or hybrid generation	Distribution of seedlings according to their reaction to form 21												Total No. F_2 seedlings
	0	1−	1	1+	2−	2	2+	3−	3	3+	4−	4	
Marquis								3	17	32	119	24	195
Vernal		82	95	19									196
F_1				1	12	5							18
F_2	9	38	60	49	43	36	17	14	29	27	33	12	367

Reaction of F_1 and F_2 Seedlings to Form 21

As a preliminary to the test of progeny of F_2 plants, several hundred F_2 seeds as well as a few F_1 seeds and seeds of both parent varieties, were sown in the greenhouse for testing to form 21. The results are given in Table I. The F_1 seedlings showed in this test reactions varying from 1+ to 2, demonstrating the partial dominance of resistance to form 21 (Fig. 2, Plate III). The F_2 seedlings fell into two main groups, one with varying degrees of resistance and the other with varying degrees of susceptibility. The seedlings of the susceptible parent variety gave reactions 3 to 4 with the exception of three (1.5% of the total) which is well within the limits of experimental error. Considering then that reactions 3 to 4 cover the range of susceptibility, there were 101 susceptible F_2 seedlings in a total of 367 plants. The agreement of the observed numbers with the expected on the basis of a single factor difference is fairly good, as shown in Table II. If, however, the F_2 seedlings that reacted 3− are apportioned equally to the resistant and susceptible classes the fit to a 3 : 1 ratio is still satisfactory.

TABLE II

SEGREGATION OF VERNAL × MARQUIS F_2 SEEDLINGS TESTED TO FORM 21 IN THE GREENHOUSE IN 1927–1928

Rust Class	Observed	Expected according to the ratio 3 : 1	Dev. / P.E.
Resistant or partially resistant	266	275.25	1.65
Susceptible	101	91.75	

Reaction of F_3 Progeny to Form 21

Of the remaining F_2 seeds, 2,500 were sown in the nursery in 1927. As might be expected in a cross between wheats of unlike chromosome number, there was poor germination of F_2 seeds. Unfavorable soil conditions added to the elimination of F_2 individuals, and only 1,251 (50%) of them survived to the seedling stage. In the Marquis check rows 69.2% of the seeds sown produced seedlings; in Vernal the percentage was 71.2. The mortality in the hybrid material was high, only 852 plants reaching the heading stage. A heavy epidemic of stem rust was present in the nursery soon after this and the susceptible F_2 plants were harvested immediately. A random group of 234 plants, part of them being among those harvested soon after heading, was studied for various characters. Many of these plants produced too few seeds to allow satisfactory greenhouse tests of F_2 progeny, consequently only 85 progeny were tested for seedling reaction.

Since the susceptible F_2 plants were harvested at about heading time, they developed no seed and their genetic constitution with respect to resistance to form 21 could not be determined by a progeny test in the greenhouse. It was observed in the field, however, that there was a definite lack of intermediates between plants which were resistant and those which were susceptible. Moreover, from rust collections made at different points in the nursery and sent to the Dominion Rust Laboratory, Winnipeg, for identification, form 21 was found to be the one that was most prevalent in the nursery. This form was one of the four isolated from collections taken from Vernal × Marquis F_2 plants. The field reaction was therefore due, in part at least, to form 21.

When the progeny of F_2 plants, which had been allowed to mature in the nursery, were tested to form 21 in the greenhouse, it was found that they were either homozygous for resistance, or heterozygous, with the exception of two that were susceptible (V 91 and V 191). The results are presented in Table III. The range of reaction of seedlings of resistant progenies to form 21 was somewhat greater than that of the resistant parent variety. This was to be expected since morphological and physiological factors may modify the expression of the main gene or genes governing rust reaction. In the heterozygous progenies, seedlings with rust reactions from 3 to 4+ may be classed as

susceptible. The numbers of resistant and susceptible seedlings agreed fairly well with the expected numbers on the basis of a 3 : 1 ratio, as shown in Table IV.

TABLE III

THE GREENHOUSE REACTIONS TO FORM 21 OF SEEDLING PROGENY OF VERNAL × MARQUIS F_3 PLANTS GROWN TO MATURITY IN THE NURSERY IN 1927

Parent variety or hybrid generation	Distribution of seedlings according to their reaction to form 21													No. of progenies*
	0	1−	1	1+	2−	2	2+	3−	3	3+	4−	4	4+	
Resistant F_3	8	106	177	98	18									24
Heterozygous F_3	5	91	197	195	123	70	14	12	44	59	130	17	2	59
Susceptible F_3									1	3	17			2
Vernal		83	285	55										
Marquis									12	66	284	22		

Only progenies with results on at least eight seedlings were considered.

TABLE IV

SEGREGATION OF F_3 SEEDLINGS FROM HETEROZYGOUS VERNAL × MARQUIS F_3 PLANTS FOR REACTION TO FORM 21 IN THE GREENHOUSE

Rust class	Obtained numbers of F_3 seedlings	Expected results on the basis of a 3 : 1 ratio	Dev./P.E.
Resistant or partially resistant	707	719.25	1.36
Susceptible	252	239.75	

V 91 and V 191, the two susceptible F_2 plants mentioned above, were interesting. When the mature plants in the nursery were examined for rust infection these two plants were the only ones in the part of the population studied that had "5"[1] (susceptible) pustules. Although Marquis controls examined at the same time had 70% infection with "5" pustules, one of these plants, V 191, showed only 15%. Probably some morphological or physiological influence had hindered infection of this plant. Infection of F_3 seedlings of V 191 was not recorded as being different from that of the Marquis controls. V 91, the other susceptible plant, showed 50% infection with "5" pustules. It was extremely late in maturing and, at the time when all the clearly susceptible plants were removed from the nursery, this plant had probably not headed and was at a comparatively resistant stage of development. The writers (13) and others have found that it is comparatively difficult to obtain extensive infection of plants of susceptible wheat varieties during the period between the seedling and heading stage.

[1] *See footnote to Table V.*

Reaction of Hybrids to Several Rust Forms in the Nursery

Infection in the nursery in 1927 was remarkably uniform and heavy on the Marquis controls. From rust cultures sent to the Dominion Rust Laboratory, Winnipeg, for identification, it was found that in addition to form 21, forms 17, 29, 36 and C (26) were present in the nursery. The reactions of the F_2 hybrid plants were very distinct. In a random sample of 234 F_2 plants 165 were resistant or partially resistant and 69 were susceptible, including V 91 and V 191. Fitting these figures to a 3 : 1 ratio gave $\frac{\text{Dev.}}{\text{P.E.}} = 1.58$. The fit is good and indicates that a single main genetic factor governs the reaction to all of the forms of rust in the nursery.

Relation between Reaction to Several Forms in the Nursery and the Reaction to Form 21 in the Greenhouse

The greenhouse tests of F_3 seedlings to form 21 divided the F_2 plants into three groups, resistant, heterozygous, and susceptible, with respect to reaction to that form. Since, in the field, resistance was found to be dominant with a single main genetic factor governing the reaction to a number of rust forms, no attempt was made to distinguish between the resistant and heterozygous plants, although the reaction of each plant was recorded in terms of the character of the pustules as well as in percentage infection. The question then arose, was there a perceptible difference in reaction between resistant and heterozygous plants in the field, or were these plants quite indistinguishable in their reactions? To settle this point the field reactions and the seedling reactions to form 21, one of the most prominent forms in the field, were studied. The results are given in full in Tables V and VI.

TABLE V

THE REACTION OF VERNAL × MARQUIS F_2 HETEROZYGOUS PLANTS TO SEVERAL FORMS OF STEM RUST IN THE NURSERY AND THE REACTION OF SEEDLING PROGENY OF THESE PLANTS TO FORM 21 IN THE GREENHOUSE

F_2 plant number	Nursery reaction		Distribution of F_3 seedling progeny according to their reaction to form 21													Seedlings totalled by rust classes	
	Character* of pustules	Per cent ** of rust present	0	1−	1	1+	2−	2	2+	3−	3	3+	3−	4	4+	R.	S.
11	2	4		2	1	1	4	1				1	1			9	2
13	1	2		1	2	2	2	2				3	2			9	5
14	1	1		2	1	3	4	3	2		1		2			15	3
15	3	7			6	4	2	1	1	2	2		5			16	7
16	2	2		1	7	3	4	3	1		1					19	1
19	2	3		2	4	2	4	3			1	4	2			15	7
23	4	25		4	3	2	1					1	1			10	2
25	1	1		2	2		1	2			1	1	1			7	3
26	2	4		5	2	2	2		1			1	2			12	3
27	3	8		1	4	1	2	2			1	1	2			10	4
31	2	4		3	4	4	6	2	1			3	6			20	9
36	2	4		1	6	4	1	1					3			13	3
41	3	9			2	1	1	2		1	1	3				7	4
45	4	35		1	2	6	4	1	1	1	2					16	2
72	4	15		3	4		1	1					1			9	1
112	3	7			2	5	3	4			1	3	4	2		14	10
115	2	20	1	6	4	5	1	2			2	2	3	2		19	9
116	4	30	2		6	3	2				1		3	1	1	13	6
117	4	25		1	5	2	1				2	3	5			9	10
120	2	15		3	4	6	2	3			1		2	2		18	5
122	2	6	1		4	4	1	1	1				2			12	2
123	2	2	1	3	6	1		1			1	1		1		12	3
127	2	2		2	3	1	1				1	1	1			7	3
128	3	8		1	2	4	2	1				1				10	1
167	1	1		1	1	4	4	1			1	1	1			11	3
169	2	3		3	4	6	2				1	1	2			15	4
172	2	4		6	5	2				1	1	4	1			14	6
173	2	4			1	2	2				1		2			5	3
174	2	6		3	2	6	3	1			1	2	4			15	7
175	1	4		6	5	5						2	5			16	7
176	3	7		2	7	8	2				1		3	1		19	5
178	3	15			1	2	1	2					5	1		6	6
182	2	5		2	1	2	2	1					1			8	1
190	1	1			3	6	2					1	2			11	3
192	3	15			5	2	2					2	2			9	4
266	1	1		4	5	9	2				2	1	2			20	5
268	2	4			4	3	2					2	3			9	5
269	1	1		5	1	2	5	1			2		2	1	1	14	6
270	3	6		1	4	2							4			7	4
271	4	30			3	4	2	3		2	3	3	1			14	7
272	3	30		3	2	2						1	1			7	2
273	3	25			2	1	1	1				1		1		5	2
275	2	10		1	4	12					1		2			17	3
281	2	6		3	3	1	2						4	1		9	5
282	2	8			3	7	3				2					13	2
283	4	35			2	3	2					1	1			7	2
285	2	20			3	1	4	4	1		1	1	1			13	3
286	1	1		2	5	3	1						3			11	3
287	2	15			2	3	3	4	1			1	1			13	2
288	2	4			3	2	5	3				2	4			13	6
292	2	4			3	6	6	3			1	1	4			18	6
293	3	10			1	4	2		1	1			2			9	2
294	2	8				3	2	2			1		3	1		7	5
297	3	7		1	4	2		2	1	1			3	2		11	5
299	2	8		1	5	5	3	1					5	1		15	6
301	1	6			3	2	2	3	1	2	5	2	2			13	9
302	2	2		2	2	2	2						4			8	4
306	3	3		1	9	4	1	2	1				1			18	1
308	2	4			3	1	1			1	1	1	1			6	3
Totals for 59 heterozygous F_2 plants			5	91	197	195	123	70	14	12	44	59	130	17	2		

Character of pustules: 1— weak, narrow pustules; 2— mid-weak, mid-narrow pustules; 3— moderately developed pustules of moderate width; 4— mid-strong mid-wide pustules; 5— strong, wide pustules.

***Per cent of rust present = area of culm occupied by pustules.*

TABLE VI

THE REACTION OF VERNAL × MARQUIS F_2 RESISTANT PLANTS TO SEVERAL FORMS OF STEM RUST IN THE NURSERY AND THE REACTION OF SEEDLING PROGENY OF THESE PLANTS TO FORM 21 IN THE GREENHOUSE

F_2 plant number	Nursery Reaction		Distribution of F_3 seedling progeny according to their reaction to form 21										Seedlings totalled by rust classes	
	Char.* of pustules	Per cent of rust present**	0	1−	1	1+	2−	2	2+	3−	3	3+	4−	4	4+	R.	S.
17	2	3		5	8	3										16	
29	2	5		6	13	10										29	
33	1	1		3	12	7										22	
35	2	2		6	4	3	1									14	
47	2	3			2	3	3									8	
76	1	1			12	1										13	
105	1	1	1	4	7											12	
113	2	12	2	8	1	2	2									15	
114	2	10	2		8	7										17	
118	2	20		5	6	5	2									18	
125	1	2	1	12	6	1										20	
171	2	8			5	6	2									13	
181	2	4		4	9	2										15	
187	2	2		1	13	2										16	
263	3	10		1	4	8										13	
265	2	9			4	13	3									20	
278	2	5		12	9											21	
284	3	25		10	7	1										18	
289	3	25		2	8	11	2									23	
291	1	3		5	6											11	
296	2	2	1	12	7											19	
303	1	1		1	11	5										17	
304	1	1	1	9	8											18	
307	2	10			7	8	3									18	
Totals for 24 resistant F_2 plants			8	106	177	98	18										

**Character of pustules:* 1− *weak, narrow pustules;* 2− *mid-weak, mid-narrow pustules;* 3− *moderately developed pustules of moderate width;* 4− *mid-strong, mid-wide pustules;* 5− *strong, wide pustules.*

***Per cent of rust present = area of culm occupied by pustules.*

In Table VII these results are summarized and a comparison made between the yield results of the form 21 heterozygotes and resistants. The 59 heterozygotes averaged 2.31 ± 0.08 for the character of the pustules. The 24 resistant plants averaged 1.83 ± 0.09. The difference of 0.5 ± 0.1 appears to be statistically significant. When percentage rust infection is considered, a less striking difference (2.5 ± 1.3) is obtained. These results indicate a measurable difference between the heterozygotes and resistants with respect to their pustule character in the field, but show no significant difference in percentage of rust infection. In general the most resistant plants in the field proved to be homozygous for resistance to form 21 when tested in the greenhouse. The probability that the same genetic factor is responsible for both the field resistance and the seedling resistance to form 21 is strongly suggested.

TABLE VII

COMPARISON OF THE NURSERY REACTIONS OF VERNAL × MARQUIS F_2 PLANTS THAT PROVED TO BE HETEROZYGOUS OR RESISTANT, RESPECTIVELY, IN THE SEEDLING STAGE, TO FORM 21

No. of F_2 plants	Reaction to form 21 in seedling stage	Reaction in nursery where several forms of rust were present					
		Character of pustules			Percentage of infection		
		Minimum	Maximum	Mean	Minimum	Maximum	Mean
59	H	1	4	2.31 ± 0.08	1	35	9.4 ± 0.8
24	R	1	3	1.83 ± 0.09	1	25	6.9 ± 1.0
	Diff. H − R			0.5 ± 0.1			2.5 ± 1.3

Relation between Field Pustule Character and Field Rust Percentage

In view of the apparently significant difference between the field rust reaction of the form 21 "heterozygotes" and "resistants" it is of particular interest to examine further the observations taken in the field on the types of rust. Both the character of the pustules and the percentage of the culm covered by the pustules were recorded. Since character of pustule is not usually observed in field studies of this kind, it is important to ascertain its value, if any, when percentage of rust is also taken. Accordingly the relation between the field pustule character and field rust percentage was calculated for the 59 heterozygous F_2 plants. A correlation coefficient of 0.74 ± 0.04, indicating a close relation, was obtained.

A closer scrutiny was then made by comparing the mean rust percentage of all plants showing pustules of character 1 with that of the plants with character 2, and so on. The results of this comparison appear in Table VIII. Plants with character 1 had a mean rust percentage of 1.90 ± 0.23 and those ith character 2 showed 6.5 ± 0.6, the difference being 4.6 ± 0.7. The odds are very high that this difference is significant. From the point of view of pustule character each group of plants differed from each of the others in percentage of rust. Apparently either a note on pustule character or on rust percentage expresses fairly accurately the degree of resistance of a plant in the field.

TABLE VIII

VERNAL × MARQUIS F_2 PLANTS WITH VARYING PUSTULE CHARACTERS IN THE FIELD COMPARED FOR PERCENTAGE RUST INFECTION

Pustule character	Mean rust percentage	Pustule character	Mean rust percentage	Difference between means
1	1.90 ± 0.23	2	6.46 ± 0.64	4.6 ± 0.7
1	1.90 ± 0.23	3	11.2 ± 1.3	9.3 ± 1.4
1	1.90 ± 0.23	4	27.9 ± 1.7	26.0 ± 1.7
2	6.46 ± 0.64	3	11.2 ± 1.3	4.7 ± 1.5
2	6.46 ± 0.64	4	27.9 ± 1.7	21.4 ± 1.8
3	11.2 ± 1.3	4	27.9 ± 1.7	16.7 ± 2.1

Reaction of Hybrids to a Number of Forms of Rust in the Greenhouse

When it was shown that a single main genetic factor was responsible in this cross for seedling resistance to form 21 it seemed probable that the same factor might be responsible for seedling resistance to a number of the forms to which Vernal is resistant. To prove or disprove this hypothesis it was desirable to test a progeny of each of a number of F_2 hybrids to each of several forms of rust in addition to form 21, if such a test had not already been made. Forms 17, 29 and 36 were available in the greenhouse, and to them Vernal is resistant and Marquis is susceptible.

Table IX represents the nature of the reactions of the parent varieties and the F_2 hybrids to a number of rust forms in the nursery.

TABLE IX

REACTIONS OF VERNAL × MARQUIS F_2 PROGENIES WHEN TESTED TO FOUR FORMS OF RUST IN THE GREENHOUSE IN 1927–1928

F_2 plant number	Rust classes of progenies when tested to:			
	Form 21	Form 17	Form 29	Form 36
V 36	H*	H		
V 125	R			R
V 172	H	H	H	H
V 174	H	H	H	H
V 175	H	H	H	H
V 266	H		H	
V 269	H	H		H
V 278	R	R		
V 451	R	R		
V 452			H	H
V 456	R	R	R	R
V 457		R	R	R
V 461		H	H	H
V 462	R	R	R	R
V 463	H	H	H	H
V 465	H	H		H
V 467		H	H	H
V 470	H	H	H	
V 471		S	S	S
V 474			R	R
V 479	H	H		H
V 480	H	H		
V 481	H	H	H	H
Vernal	R	R	R	R
Marquis	S	S	S	S

*R—*resistant*, H—*heterozygous and* S—*susceptible.*

NOTE—*Space does not permit showing all of the results; those given here are representative.*

The most important point brought out in Table IX is that all the progeny from any given F_2 plant fell into the same rust class irrespective of the form to which they were tested. When the tests to forms 17, 29 and 36 were made, the environmental conditions were very favorable for rust development. The separation of susceptible from resistant and partially resistant seedlings was even more readily made than in the tests to form 21 which were

summarized in Table III. The rust class in which each progeny should be placed was never in doubt. As an illustration of this fact, the results from a random group of resistant, heterozygous, and susceptible progenies, for each of the three forms of rust, 17, 29 and 36, are shown in Table X. The differences between the reactions of resistant, heterozygous and susceptible progenies are obvious.

TABLE X

SUMMARIZED REACTIONS OF SEEDLINGS OF A RANDOM GROUP OF RESISTANT, HETEROZYGOUS AND SUSCEPTIBLE PROGENIES TO EACH OF THE RUST FORMS, 17, 29 AND 36

Rust Class of progenies	Rust forms	Distribution of seedlings according to their reactions										
		0	1−	1	1+	2−	2	2+	3−	3	3+	4−
Resistant	17	3	41	37	6							
Resistant	29	10	32	8								
Resistant	36	9	31	12								
Heterozygous	17	1	19	21	17	25	17	4	?	9	21	10
Heterozygous	29	4	15	27	35	19	12	3	4	18	15	7
Heterozygous	36	2	12	11	9	11	7	2	2	4	8	2
Susceptible V 471	17									4	9	4
Susceptible V 471	29									1	6	2
Susceptible V 471	36									5	11	4
Vernal	all	7	40	11								
Marquis*	all									10	34	20

**Since environmental conditions were very favorable, it was possible to save time by recording infection at a shorter interval (14 days) after inoculation than is usually advisable. The range of reaction of Marquis in this test was from 3 to 4− while usually it is from 3+ to 4.*

It is therefore reasonable to conclude that as the reaction of progeny of any F_2 hybrid to any of the four forms of rust falls into the same rust class, the factor in Vernal which is responsible for resistance to form 21, is the same factor that governs resistance to forms 17, 29 and 36.

Reaction of F_1 and F_2 seedlings to form 27.

A reciprocal relation exists between the reactions of Vernal and Marquis to forms 21 and 27, Vernal being moderately susceptible and Marquis resistant to the latter form. In order to learn more about the inheritance in the cross Vernal × Marquis, hybrids were tested to form 27, with results as shown in Table XI.

TABLE XI

THE REACTIONS OF F_2 SEEDLINGS OF VERNAL × MARQUIS TO FORM 27 IN THE GREENHOUSE IN 1927–1928

Parent variety or hybrid generation	Distribution of seedlings according to their rust reaction											
	0	1−	1	1+	2−	2	2+	3−	3	3+	4−	4
F_1				3	4	4						
F_2	43	35	34	37	25	32	10	11	17	28	13	6
Vernal								8	45	95	7	
Marquis	12	40	31	6								

The reaction of the F_1 seedlings indicated the partial dominance of resistance. (Fig. 2, Plate III). The F_2 seedlings seemed to consist of two main groups, one being fully as susceptible as Vernal and the other group being more or less resistant with the dividing line between reactions 3− and 2+. The F_2 results provided a good fit to a 3 : 1 ratio, as shown in Table XII.

TABLE XII

SEGREGATION FOR RESISTANCE AND SUSCEPTIBILITY OF VERNAL × MARQUIS F_2 SEEDLINGS INOCULATED WITH FORM 27

Rust classes	Obtained number of seedlings	Expected number according to 3 : 1 ratio	Dev. / P.E.
Resistant or partially resistant	216	218.25	0.45
Susceptible or moderately susceptible	75	72.75	

Notwithstanding the close fit of the F_2 results to a monohybrid ratio there were indications that more than a single genetic factor concerns resistance to form 27. The proportion of F_2 seedlings with 0 reaction was much larger than was expected, more hybrids having reaction 0 than 1−, whereas in the resistant parent variety only 12 plants showed a 0 reaction and 40 showed 1−. Similarly there were more susceptible hybrids than were expected. Nineteen out of 85 F_2 plants showed 4− and 4, but only 7 out of 153 plants of Vernal, the susceptible parent variety, gave a 4− reaction and none gave 4.

Reaction of F_3 Seedlings to Form 27

F_3 progenies were then tested to this form to determine more accurately the nature of the inheritance of the reaction. Owing to poor environmental conditions in the field and to the partial sterility of many plants, less than half of the F_2 mother plants had produced sufficient seed for adequate greenhouse tests of the F_3 progeny. As all of the F_3 progenies were tested first to form 21, only part of them had enough seed for an adequate test to form 27. The 57 that were tested, however, constituted a random sample except with respect to plant vigor and fertility. Some of the results are given in Table XIII, and will be discussed in connection with the results from the form 21 tests.

Genetics of the Reaction to Forms 21 and 27

The distribution of seedlings of F_3 families for reactions to forms 27 and 21, suggested two factors for resistance, a dominant factor R_b, in Vernal, producing high resistance to form 21 and slight resistance to form 27, and a dominant factor R_a in Marquis, causing resistance to form 27. It was assumed that R_a did not affect reactions to form 21. The constitution of Marquis would then be $R_aR_ar_br_b$ and of Vernal $r_ar_aR_bR_b$. The genetic constitution of the mother plant of each F_3 family was then determined. The condition of factor R_b for each F_2 plant was determined from the reaction of its progeny to form 21, a

TABLE XIII

REPRESENTATIVE RESULTS FROM TESTS OF PROGENIES OF VERNAL × MARQUIS F_2 PLANTS TO FORM 27; AND THE ASSUMED GENETIC CONSTITUTIONS OF THE F_2 PLANTS WITH RESPECT TO FACTORS R_a AND R_b

F_2 plant number	Distribution of F_3 seedlings according to their reaction when inoculated with form 27													Form 21 rust class	Condition of R_b	Form 27 rust class	Condition of R_a	Constitution with respect to R_a and R_b
	0	1−	1	1+	2−	2	2+	3−	3	3+	4−	4	4+					
V 25	15	1	3	1	1	2								H*	R_br_b	HR*	R_aR_a	$R_aR_ar_br_b$
V 26	4	1	2	3		2				1	1	5	1	H	R_br_b	H	R_ar_a	$R_ar_aR_br_b$
V 27	3	2	1	2	2	4	1							H	R_br_b	HR	R_aR_a	$R_aR_ar_br_b$
V 29**	6	4	3	1	1									R	R_bR_b	R	R_aR_a	$R_aR_aR_bR_b$
V 31				3	1	4	1			1	4	2	1	H	R_br_b	HI	R_ar_a	$R_ar_aR_br_b$
V 33	4	2	3	2		1	2			2	1			R	R_bR_b	H	R_ar_a	$R_ar_aR_bR_b$
V 35	5	4												R	R_bR_b	R	R_aR_a	$R_aR_aR_bR_b$
V 36	1					1	7			1	1			H	R_br_b	H	R_ar_a	$R_ar_aR_br_b$
V 41										1	5	3		H	R_br_b	HS	r_ar_a	$r_ar_aR_br_b$
Vernal								4	23	24	2			R	R_bR_b	IS	r_ar_a	$r_ar_aR_bR_b$
Marquis	19	9	3	3	2	8	3							S	r_br_b	IR	R_aR_a	$R_aR_ar_br_b$
V 291						1	2	3	2	1				R	R_bR_b	IS	r_ar_a	$r_ar_aR_bR_b$
V 292							1	2	1	1	3			H	R_br_b	HS	r_ar_a	$r_ar_aR_br_b$
V 293	1	1		2		3		2		1	3	2		H	R_br_b	H	R_ar_a	$R_ar_aR_br_b$
Vernal								20	21	6	1			R	R_bR_b	IS	r_ar_a	$r_ar_aR_bR_b$
Marquis	16	9	5	1	4	6	2							S	r_br_b	IR	R_aR_a	$R_aR_ar_br_b$

* R = *resistant*; HR = *Heterozygous resistant*; H = *Heterozygous*; HI = *Heterozygous intermediate*; IR = *Intermediate resistant*; IS = *Intermediate susceptible*; HS = *Heterozygous susceptible*; S = *Susceptible*.

**NOTE: *There was insufficient seed of V 28, 30, 32, 34, 37–40 for tests to form 27 after the tests to form 21 had been made.*

resistant plant being R_bR_b and a heterozygous one R_br_b. The distribution of seedlings in a separate progeny, tested to form 27, determined the F_2 plant condition with respect to factor R_a. The form 27 results for 12 representative progeny together with the rust classes and theoretical genetic constitution of the mother plants are shown in Table XIII.

It will be observed in Table XIII that the numbers of seedlings in progenies are in many cases so small that complete verification of the hypothesis at this time is not possible. It is also apparent that the range of reaction of the Marquis control in this test is wider than that of the Marquis control for the F_2. The Marquis control of the F_2 was strain Sask. 70 and of the F_3 was Sask. 7. Marquis Sask. 7 consists of two main morphological types called B and C and a small proportion of intermediates (12). The relatively wide range of reaction of Marquis 7 suggested that the two types within the strain reacted differently to form 27. Consequently, seedlings of Marquis 7, of its two main types 7B and 7C, and of Marquis 70, a uniform strain, were tested to this form. Results shown in Table XIV indicate that the two types do not give the same reaction. The 7B type had a reaction range from 2 to 3, whereas the 7C type showed reactions 1 to 2−. Marquis 70 also had a range from 1 to 2−. The C type of Marquis 7 has been found to be typical of this variety whereas the B type is not.

TABLE XIV

TESTS OF SEEDLINGS OF MARQUIS 70, MARQUIS 7 AND THE TWO TYPES OF MARQUIS 7 TO FORM 27 IN THE GREENHOUSE, 1927–1928

Seedlings tested	Distribution of seedlings according to rust reaction						
	1	1+	2−	2	2+	3−	3
Marquis 7B				1	7	10	8
Marquis 7C	10	10	7				
Marquis 7	5	7	8	3	3	7	5
Marquis 70	6	16	11				

Variation in the pathogenic reaction of different strains within a commercial variety is not uncommon. Two strains of Hannchen barley consistently react in quite a different manner to *Helminthosporium gramineum* at Saskatoon. Recently Kiesselbach and Peltier (19) found "that there may be decided variation in the differential reaction of wheat strains within a commercial variety to various physiologic forms of *Puccinia graminis tritici*".

The variability of Marquis 7 makes the analysis of the data more difficult. The 7B type comprises about 34% of Marquis 7 (12), therefore it is reasonable to assume that this type was the male parent of some of the F_1 plants produced. The distribution of seedlings in a number of F_3 progenies can be explained readily on this basis. V 31, shown in Table XIII, may be used as an example. If the Marquis parent were of the 7B type, transgressive segregation, such as would be expected from a plant of the genotypic class $R_ar_aR_br_b$, would give a

distribution similar to that shown by V 31. The resistant seedlings showed rust reactions corresponding with the *less* resistant half of the Marquis 7 range, as expected from the results in table XIV. On the other hand, the distribution of seedling reactions in some families in the test to form 27, did not seem to correspond closely with the genetic constitution that was indicated by the test to form 21. For example, in the progeny of hybrids V 29 and V 291, which are assumed to be homozygous for R_a and R_b and for r_a and R_b, respectively, the range of reaction of the seedlings is wider than might be expected. However, although they may be homozygous for these factors, it is probable that they are heterozygous for factors which modify the expression of the main factors. For example, if the resistance factor, termed R_a, which governs reaction to form 27, expresses itself morphologically, and if hundreds of genetic factors in a hybrid affect the same morphological features, variability in the reactions of hybrid progeny must be expected. Then too, environmental conditions may have caused some of the variation, as evidenced by the distribution of the control seedlings.

TABLE XV

THE GOODNESS OF FIT OF THE OBSERVED RESULTS WITH THE CALCULATED FIGURES FOR THE TESTS OF F_2 PROGENIES OF THE CROSS VERNAL × MARQUIS TO FORMS 21 AND 27 IN THE GREENHOUSE

Genotypic classes	Expected ratios	No. of F_2 progenies		O−C	$(O-C)^2$	$\frac{(O-C)^2}{C}$
		Observed	Calculated			
$R_aR_aR_bR_b$	1	8	5.42	2.58	6.656	1.228
$R_ar_aR_bR_b$	2	11	10.83	0.17	0.029	0.003
$R_aR_aR_br_b$	2	10	10.83	0.83	0.689	0.064
$R_ar_aR_br_b$	4	25	21.67	3.33	11.089	0.512
$r_ar_aR_bR_b$	1	4	5.42	1.42	2.016	0.372
$r_ar_aR_br_b$	2	7	10.83	3.83	14.669	1.354

$X^2 = 3.533$ P. = 0.62

On the whole the two-factor hypotnesis fits the results remarkably well. This is shown clearly in Table XV where the results are fitted to the expected figures by the X^2 method. Progenies of less than eight seedlings were not included in the table. The factorial combinations $R_aR_ar_br_b$, $R_ar_ar_br_b$, $r_ar_ar_br_b$ do not appear in the summary, for, as explained previously, all hybrids homozygous for factor r_b, were susceptible to form 21 and therefore had been removed from the nursery soon after the heading stage. Progeny of these plants could not be tested in the greenhouse as they produced no seed.

Height of plant

Since the part of the population that was susceptible to stem rust in the nursery had been harvested before maximum growth was reached, the height of these plants could not be taken as a true index of the height they might have attained. A study (34) had been made by the junior author of a number of hybrids with respect to height at heading time and at maturity. It had been found that on all hybrids of which the spike of the first culm had begun to emerge, the distance from the crown to the top of the highest leaf sheath could be used as a measure of relative height. It was therefore possible to determine the relation between the height of the plant and other characters which had been studied.

Relation between characteristics

Investigations have been made by Sax (33) and Thompson (36) in which the relation between rust reaction and other characters has been studied. A close correlation was reported between the *durum* resistance to rust and other *durum* characteristics. A study of hybrids of *T. dicoccum* × *T. vulgare* was made by Hayes, Parker and Kurtzweil (15), but the number of plants used was not large. They reported a correlation between *dicoccum* rust resistance and *dicoccum* head type. On the other hand, Hynes (18) found that in hybrids of a cross between Federation (*T. vulgare*) and Khapli (*T. dicoccum*) there appeared to be no relation between head type and rust reaction.

The importance of these relations in the work of combining emmer rust resistance with bread wheat characters is apparent. In the present investigation, the general type of the hybrid has not been considered, but two morphological features in which the parent varieties differ markedly, and some important economic characteristics, have been studied.

The coefficient of contingency as described by Hayes and Garber (14) was used in the statistical study of the relationships between characters. This was done because most of the data were recorded in categories and because numerical data, where available, were not distributed strictly according to a normal frequency curve. The various relationships are shown in Table XVI.

TABLE XVI

DEGREE OF ASSOCIATION OF CHARACTERS IN F_2 HYBRIDS OF VERNAL × MARQUIS

Characters	Contingency coefficient C_1
Resistance to form 21 and adherence of glumes	0.3 ± 0.1*
Resistance to form 21 and emmer seed shape	0.3 ± 0.1
Resistance to form 21 and resistance to form 27	0.4 ± 0.1
Resistance to form 27 and adherence of glumes	0.4 ± 0.1
High fertility and adherence of glumes	0.30 ± 0.09
High fertility and emmer seed shape	0.37 ± 0.09
High fertility and tallness	0.70 ± 0.05

**The probable error of the coefficient of contingency was obtained by the formula:*

$$\text{P.E.} = \frac{2 \times 0.6745\,(1-C_1^2)}{\sqrt{n}}$$

NOTE: *The number of F_2 plants* (n) *involved in these relationships averaged 134.*

These associations will be discussed briefly. The relation between seedling resistance to form 21 and seedling resistance to form 27, shown by the contingency coefficient 0.4 ± 0.1, was weak as was expected according to the hypothesis that the factor R_b for high resistance to form 21 also brings about slight resistance to form 27. There appeared to be a significant though weak relation ($C_1 = 0.4 \pm 0.1$) between resistance to form 27 and adherence of glume to the seed, but no reason for it was apparent. The close correlation between high fertility and plant tallness ($C_1 = 0.70 \pm 0.05$) was to be expected, since the tall plants were usually the most vigorous and tended to have high percentages of florets setting seed.

Several investigators (20, 33, 37) have found that in crosses between varieties of *T. vulgare* and varieties of *T. durum* or *T. dicoccum* a large proportion of the hybrids have the chromosome number of the *durum* or the *dicoccum* parent variety. It would be expected that these hybrids would have moderately high fertility. In the present study the relation was determined between high fertility and two characteristics, adherence of the glumes to the seed, and seed shape, which are economically important in comparisons of *T. dicoccum* with *T. vulgare*. The contingency coefficients are 0.30 ± 0.09 and 0.37 ± 0.09, respectively. Although the coefficients appear to be significant in each case they evidently indicate weak relationships.

Nothing definite was learned about the degree of association between resistance to form 21 (which is also resistance to forms 17, 29 and 36) and the *dicoccum* characters adherence of glume to the seeds and emmer seed shape owing to the large probable errors of the contingency coefficients. It seemed probable, however, that if these characteristics had been correlated to any extent the coefficients of contingency would certainly have revealed the relations, for there were 126 pairs of results in each case. It appears evident that no marked relation exists between Vernal rust resistance and the other characteristics of Vernal that were studied in the Vernal × Marquis hybrids.

Discussion

The Mendelian Inheritance of Resistance in a Species Cross

Some results of major importance in the study of the genetics of species crosses and of rust reaction in particular have been obtained in this investigation. In the cross, Vernal (*T. dicoccum*) × Marquis (*T. vulgare*) the existence of a single main genetic factor governing the reaction to four forms of stem rust has been demonstrated. The simple monohybrid segregation is noteworthy in view of the fact that the parent varieties have different chromosome numbers and that, in crosses between them, chromosome behavior is irregular in the formation of gametes. Previous studies on the inheritance of rust reaction in wheat crosses between species with fourteen and with twenty-one pairs of chromosomes respectively have not shown a well defined monohybrid ratio. Puttick (29) found indications of a single factor pair governing rust reaction in a *durum* × *vulgare* cross, but the segregation was not clear-cut and only F_2 results were reported. The typical Mendelian inheritance of the factor pair $R_b r_b$, governing reaction to form 21 in the present study, shows that it is located in chromosomes that mate regularly when Vernal and Marquis are crossed.

Objection to this statement might be raised on the ground that the population studied was not random. It is true that relatively few of the possible gametic combinations get as far as the seed stage owing to lethal chromosomal irregularities. Only 33% of the F_2 seeds sown in the nursery produced plants that headed and could be considered for rust reaction. Of these plants, some produced so few seeds that all the F_3 progeny tested to form 21 in the greenhouse represented only 18% of the seeds sown in the field. The group of plants studied was not random, therefore, with respect to endosperm development, plant vigour, seed setting, or any other character associated with zygotic mortality. But this zygotic elimination need not be considered here unless it is associated with the genetic factors involved in rust reaction, the chromosomes that carry them, or the particular individuals in which these chromosomes occur. Such a connection or relation has not been demonstrated as far as the writers have ascertained.

Seedling vs. Field Resistance

Field resistance is not necessarily governed by the genetic factors that control seedling resistance. Aamodt (1) discovered that a variety might be resistant to several forms of rust in the field yet be susceptible to one of these forms when tested in the seedling stage. Goulden, Neatby and Welsh (9) found that H-44-24 × Marquis F_3 families, which bred true for susceptibility to forms 21 and 36 in the seedling stage, segregated for reaction in the field, where the same forms of rust were known to be present. In the present study the evidence suggests that the single main genetic factor, R_b, which governs the reaction of seedlings to several forms of rust in the greenhouse, also controls the field reaction to a number of forms including those which were used in the greenhouse.

The Nature of Resistance

The presence of a single main genetic factor for high resistance to a number of forms of stem rust is of much importance from the standpoint of the production of a desirable bread wheat which is resistant to rust. The possibility is suggested that the same factor determines both the high field resistance of Vernal to 35 of the known forms of stem rust and the moderate field resistance of that variety to the remaining forms. Hursh (17) has presented evidence tending to show that the low infection of Vernal by rust is more morphological than physiological. He found that sclerenchymatous tissues made up the major portion of the stem in Vernal and formed a mechanical limitation to the spread of rust mycelium. Leaves of seedlings were found to have less sclerenchymatous tissue development than older leaves or stems. Therefore, while resistance to stem rust may be fundamentally physiological, seedling tests in the greenhouse might frequently be a poor indication of the field resistance of a variety; the extent of the development of sclerenchymatous tissue in the seedling might be the determining factor in the rust reaction.

Whether or not the resistance of Vernal is largely morphological or only partially so, the susceptibility in the greenhouse of seedlings of that variety to several of the known physiologic forms of stem rust is of little economic importance. The salient point is that Vernal is rarely attacked vigorously by any form of rust in the field. It is logical to suggest that in a given variety, whatever it may be that nearly always brings about complete or partial resistance to all of the known rust forms in the field, is quite likely also to involve resistance to other rust forms that may not as yet have been found, or may later be produced through the hybridization of existing forms. As far as the field reaction of Vernal and other extremely resistant varieties, such as Hope (4) and H-44-24 (8), is concerned, rust may almost be regarded as an entity. If, as the results of the present investigation suggest, a single main genetic factor controls field resistance to all physiologic forms of stem rust, the problem of obtaining this resistance factor in combination with factors for the desirable bread wheat qualities will be less complicated than it has been considered during recent years.

Infrequent Appearance of Vulgare Type

Even if rust resistance can be transferred in a simple Mendelian manner there still remain difficulties to be overcome. In *dicoccum* × *vulgare* crosses, *vulgare* types appear very infrequently. It has been pointed out that this is perhaps the greatest obstacle in transferring any *dicoccum* character into the 21-chromosome group. Sax (33) found 12 plants with 42 chromosomes in a group of 46 F_3 hybrids of the cross *vulgare* × *durum*, but Kihara (20) reported only one plant with 42 chromosomes among 20 hybrids of various crosses between 14 and 21 chromosome wheats. Thompson and Hollingshead (37) made a study of a random group of 210 F_2 plants of the cross *T. dicoccum* var. *farrum* (Percival 28) × Marquis. They found only one plant that was *vulgare* or near-*vulgare* in all of the 20 characters that they used. They studied

cytologically a random lot of 28 of these hybrids and found that the majority of the plants had 14 bivalent chromosomes, and were *dicoccum*-like in six characteristics, which they used to distinguish wheats with 14 chromosome pairs from those with 21 pairs. Rust reaction was not one of these characteristics. No plant with more than 37 chromosomes was found among these 28 hybrids, and only three of the hybrids had more than 14 bivalents. It is of much interest that these three plants (two had 15 and one had 17 bivalents) were as much like *vulgare* as like *dicoccum* in the six special distinguishing features.

From this it would seem that hybrids approaching *vulgare* in chromosome number would very largely be *vulgare*-like in all of their characters. However, this has not been proven. It is probable that, from large populations, plants would be obtained with all combinations of *vulgare* and *dicoccum* characters[1]. Sapehin (32) reports results of this nature from an extensive study of a *durum* × *vulgare* cross. He states, "Among *durum* as among *vulgare*, biotypes may be found with an equal degree of markedness of the one or the other character, compactness of the ear, character of the keel, solidity of the straw, pubescence of the leaves, etc. Thus in comparing *durum* and *vulgare* we find not one particular specific feature with which to characterize these species".

Relation between Characters

The present study indicates that the resistance of Vernal, a representative *dicoccum* variety, to a number of important forms of stem rust is inherited independently of glume adherence and seed shape, for which Vernal and Marquis differ markedly. Glume adherence has long been considered of major importance as a characteristic of *dicoccum*. It is one of the chief *dicoccum* characteristics that prevents the use of emmers in the milling and baking industries, consequently it is encouraging to find no evidence of close relation between glume adherence and rust resistance. The seed shape of emmers is, for various reasons, less desirable than that of *vulgare* wheats. The results obtained do not indicate that it would be difficult to obtain a desirable seed shape in combination with *dicoccum* rust resistance.

Size of Hybrid Population

Although the critical genetic factors for rust resistance may be inherited in a simple Mendelian manner, and although there may be no definite relation or linkage between factors responsible for desirable and undesirable characters in a *dicoccum-vulgare* cross, the fact remains that 42-chromosome hybrids emerge relatively rarely from such a cross. If a 42-chromosome wheat is the necessary objective in a practical breeding program for a desirable rust resistant variety, the plant breeder will need hundreds of 42-chromosome hybrids in the early segregating generations of a *dicoccum* × *vulgare* cross, for

[1]*This was found to be the case in 1928, when a very large F_2 population of the Vernal × Marquis cross was grown. It is expected that the results of the 1928 work will be published late in 1929.*

among the random combinations of plant characters the number of suitable types is greatly limited by the exacting requirements of the farmer, miller, baker and consuming public. Unquestionably the breeder must grow very large hybrid populations in order that there may be a reasonably good chance of attaining the goal.

The highly gratifying results obtained by McFadden (22) of South Dakota, constitute direct evidence of the value of emmer × common crosses. He crossed Yaroslav emmer, a variety of the Vernal type, with Marquis and produced the varieties Hope and H-44-24 that have almost complete resistance to rust in the field and seem to have most of the desirable qualities of Marquis. Cytological examination of H-44-24 by Elders (8) showed it to have 21 pairs of chromosomes.

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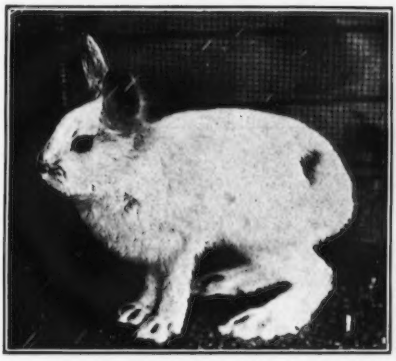


FIG. 1. *Lepus americanus in Winter Coat. Patch of white fur knocked off hip, showing dark under-fur.*

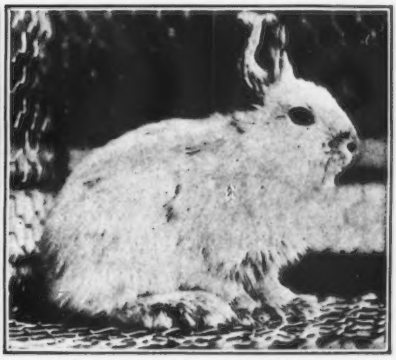


FIG. 2. *Rabbit kept indoors. Photo on March 6.*

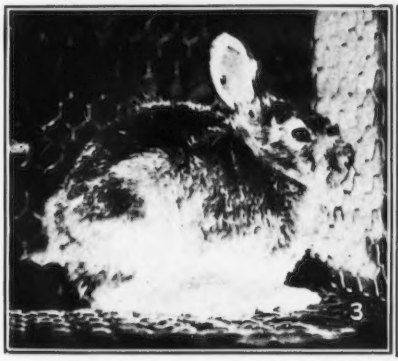


FIG. 3. *March 6. — White Outer Fur Removed by Plucking. Noticeable white guard-hairs remaining on back*

FIG. 4. *December Hair. Four bands of color and unusual thickness of white pile.*

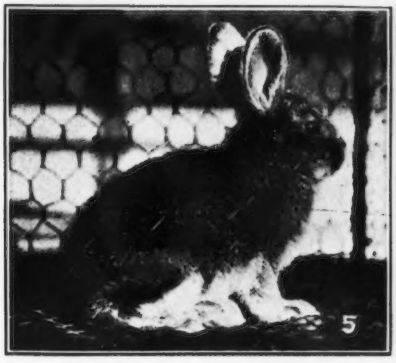


FIG. 5. *Young Rabbit Practically Unchanged.*

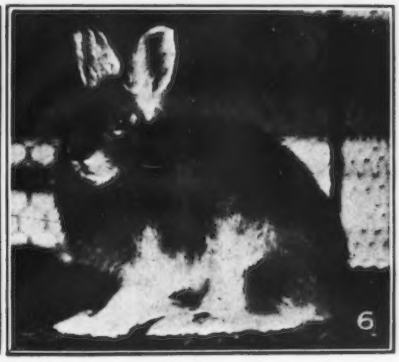


FIG. 6

(Photographs by the Author)

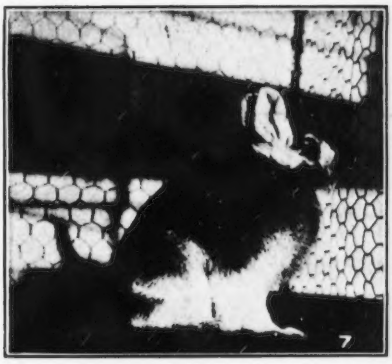


FIG. 7.

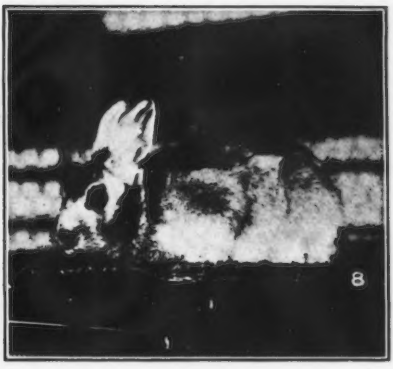
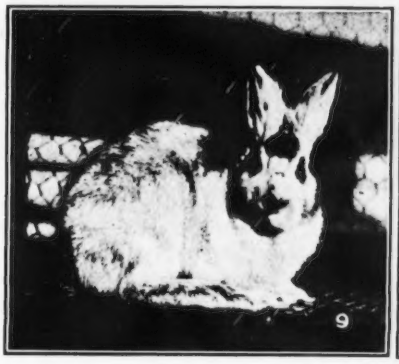


FIG. 8.



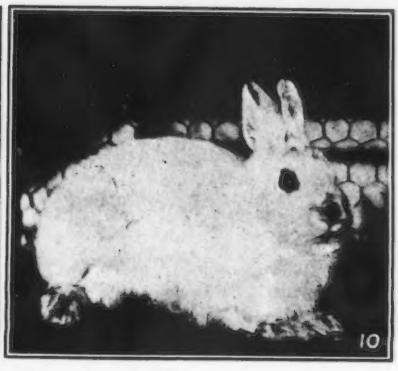


FIG. 6. (Plate IV) and FIGS. 7-10.—*Progressive Color Changes in the Rabbits.*

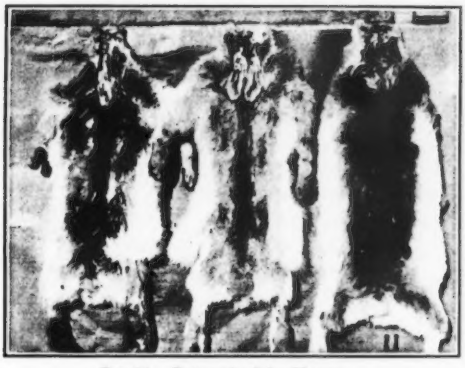


FIG. 11. *Progressive Color Changes.*

(Photographs by the Author)

COLOR CHANGES IN LEPUS AMERICANUS AND OTHER ANIMALS

BY SEYMOUR HADWEN[2]

Abstract

Observations on the color changes of *Lepus americanus* are recorded and discussed. Melanin is shown to be lacking in the white winter hair but plentiful in the dark summer hair, especially in the roots, thereby protecting the animal from sunburn. In view of the observations on experimental animals it is considered that the color changes of *Lepus americanus* are due, not to local environmental factors, but to an inherited habit. The conclusion is reached that color changes are primarily for the summer exclusion and winter retention of heat and for protection against ultra-violet light. (G.B.T.)

Introduction

In presenting the results of his work on the coloration of *Lepus americanus*, the writer feels justified in stating some of his views on the question of color in domestic and wild animals, because his point of view is that of a veterinarian who regards the color, shape, and size of animals from a health standpoint. This is not the usual point of view, especially on the part of those who are adherents to the theory of protective coloration, which the writer regards as of secondary importance.

As an example of the usual view, Dawson (4), in an exhaustive treatise on the pigmented tumors in man, made mention of aggressive coloration and protective cryptic coloration. He evidently believed that animals were quite differently constituted from man, because he said: "In animals, therefore, it is seen that pigmentation has other purposes than protection against light, heat, and moisture" How a medical man can take this attitude is hard to understand. In differing from Dawson, the writer does not pretend that his observations and experiments are complete; some of them need repetition. The present article comprises a discussion of the imperfections in white domestic animals as compared with their wild progenitors, which they may resemble outwardly, and a description of the author's experiments.

In a preliminary paper (7) the writer recalled some of the early observations made by Darwin and others on the harmful effects of sunlight on white animals, on sensitization of the skin through the ingestion of certain plants, and on the relatively high susceptibility of white cattle to parasitic attack. In a more recent paper (8) further evidence was presented showing that several species of flies, mites, and lice have a decided preference for white-skinned animals. Repetition of this evidence is unnecessary here but one of the most striking examples of the effects of sunlight and super-added parasitism may be cited. Hereford cattle possess white faces devoid of skin pigmentation. In Canada, these animals suffer both in summer and winter

[1] *Manuscript received January 4, 1929.*
Contribution from the laboratories of the University of Saskatchewan, Saskatoon, Saskatchewan, Canada.
[2] *Research Professor of Animal Diseases, University of Saskatchewan.*

from the effects of sunlight. The discharges from their eyes attract flies, resulting at times in serious trouble. In searching for an explanation for the mechanical weakness of a white skin and its attraction for insects, many skin sections have been examined. White skin is lacking in pigment-bearing cells, and due to this fact, seems more easily penetrated by parasites. Recently, through the co-operation of Professor Williams the tensile strength of 30 pieces of raw hide was determined. The pieces were cut one inch wide so as to include in each a portion of white skin. In 23 instances the skin broke through the white part although in some cases the white skin was even thicker then the pigmented portion.

These references to white domestic animals are given because unwarranted comparisons between them and the wild animals have so often been made. For instance, white reindeer are an example of the perpetuation of an inferior race of animals. They are deaf, as were also white cats with blue eyes which came to Darwin's notice, and have other defects as well. On the other hand, wild animals which turn white in winter have no such defects; as far as one can see, they are perfectly suited to their environment.

Harrington (9) commenting on the writer's observations regarding the color and health of animals (7), says: "The sun's radiation does not hurt the hair but rather the body under the hair. Black hair would absorb radiation to a high degree and thus prevent it reaching the body. The hair may get very hot, particularly on the outer surface but it is such an extremely poor conductor and a good radiator that the heat is lost by radiation rather than conducted to the body. In the other case, white hair would reflect rather than absorb and by repeated reflections from the hair surface to hair surface the radiation would in a large measure reach the body of the animal where it would be absorbed and cause discomfort." According to this hypothesis, white hair should keep the animal warmer at all times with consequent detriment to health in the summer, providing that the skin is not pigmented below it. This fits the facts observed with regard to the northern animals under discussion. Whether this physical explanation be correct or not the animals will continue to turn white in winter and dark in summer with consequent benefit to themselves. It is a distinct handicap to white domestic animals that they cannot do likewise. A white skin is unfortunately transmitted as such. Many times the writer has seen foals or reindeer fawns marked with white exactly like their mothers, showing how strongly this characteristic is inherited, even when the sire has been of a different color.

Periodic Changes of Hair Color

Spring Change of Color in the Hair.

The observations began on Dec. 24, 1927. Two rabbits (*L. americanus*), captured at Durban, Manitoba, were placed in wire cages outdoors, and three others were kept in a steam-heated room. It was expected that the rabbits kept inside would shed their white hair rapidly, but such was not the

FIG. 12. *Section taken from under Thick Hair near Eyelid. Melanin absent in skin.*

FIG. 13. *Further along in Same Section as Fig. 12. Pigmentation at edge of eyelid.*

FIG. 14. *Polar Bear Skin. Pigment cells rusty red*

FIG. 16. *Active Hair Roots during Spring Change.*

FIG. 17. *Autumn Color Change. Altered shape of hair roots and smaller amount of pigment.*

FIG. 18. *Enlarged Hair Root for Comparison with Fig. 16.*

FIG. 19. *Skin Section, Jan. 24. A few hairs show color.*

FIG. 20. *Gas Bubbles in Hair.*

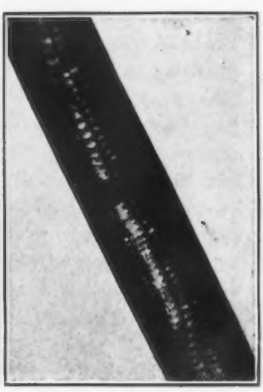


FIG. 21. *Early Color Changes in Black Hair.*

(Photographs by the Author)

case; two of these did not complete this change until the first week in May. The two rabbits outside were shedding rapidly at this time, though one of them still had a sprinkling of white pile on May 17. The shedding of the white hairs is a slow process and occurs a little at a time. It was observed that the hairs float off the rabbits in any current of air and stick to the wire meshes of the cages. Allen (1) and Nelson (11) speak of a regular moult early in the spring; but, evidently, it cannot be called more than a partial moult. This phenomenon is easily explained by an examination of the coat. The pile is tipped with white for 1.5 cm. on at the outer end, and in this zone the hairs have a width of 80 to 120μ in the widest part. At the point of contact with the brown zone below, the hairs taper down to 20-40μ in width. The hair, being brittle, breaks off at this point.

Experimental Plucking of a White Rabbit.

One of the rabbits, being very wild, frequently knocked off portions of its white fur, thereby exposing the brown underfur (Fig. 1, Plate IV). Accordingly this animal was "plucked" on March 6, 1928, and was thus changed in about half an hour from a white rabbit to a brown one.

Mechanical wear will undoubtedly shorten the moulting process and, from reports received, it seems likely that wild rabbits shed more rapidly than captive animals. In cages *L. americanus* is generally very quiet. Occasionally it may be seen rubbing its head with its forefeet. but it does not seem to be in any haste to get rid of its outer fur. After the white tips have disappeared, the first spring coat is blue near the skin succeeded by fulvous and brown; it has a length of about 2.5 cm. The winter guard hairs are still attached and overtop the coat by 1.5 to 2 cm. (Figs. 2, 3, Plate IV).

Summer Coat.

New hairs begin to show almost before all white hairs have gone. About the middle of May, the coat is colored as follows: blue, 1.3 cm., fulvous, 0.5 cm., dark brown, 0.3 cm., light yellow, 0.4 cm., and black, from 0.5-1.0 cm., making a thickness of about 3.5 cm. This coat changes but little in appearance until the middle of September. At this time it turns a shade lighter in color and some of the hairs become loose, having all the appearance of belonging to the early summer growth, the hairs of which are smaller in diameter than the September hairs. The shedding is neither very noticeable nor very profuse but continues until the hair changes to white. Even after the external coat is pure white, summer hairs may still be found. It should be mentioned that the indoor rabbits were considerably darker along the back than the outdoor animals. It is probable that this lighter coloration in the outdoor rabbits was due to the bleaching effects of sunshine.

The Autumn Coat.

Measurements were taken on four rabbits just at the period of change, Nov. 1-11. From the skin outwards, the colors were: blue, 1.5 cm., fulvous, 0.5 cm., dark brown, 0.5 cm., and the pile (changed or unchanged), 1.5 cm., making a thickness of 4 cm. (Fig. 4, Plate IV). On the sides of the abdomen the hair was 5 cm. in length.

Guard Hairs.

Though the guard hairs are the last to fall out in the spring, they are the first to change color in the autumn, becoming white several days before the pile. A possible explanation for this is given in the paragraph on pigmentation. Briefly, it may be caused by the greater depth of the roots, so that they may cease functioning before those of the pile. Furthermore, the physical aspect of the problem should not be forgotten. The colors of the winter coat would seem perfect according to the theory mentioned (9).

Autumn Changes, 1928.

Observations were made during this period on three rabbits which had been kept outside in open cages since the previous winter and on three other rabbits which had been confined in a steam heated room, two since December, 1927, and the third for a shorter period. No noticeable change occurred until September 23, when a white tinge was noticed in the hair of feet and ears. This was true of all the rabbits, whether kept indoors or outdoors (Fig. 5, Plate IV).

On Oct. 3, the rabbits indoors seemed a little whiter about the ears and feet. (Fig. 6, Plate IV).

On Oct. 23, the color changes, which up to this time had been slow and insignificant, began to occur more rapidly. One of the rabbits indoors developed white rings round its eyes and white patches at the base of the ears and on the lower part of the nose. There were also some scattered light patches on the flanks (Fig. 7, Plate V). On Oct. 27, one of the rabbits outdoors was changing rapidly; white rings had appeared around the eyes and the flanks were spotted (Fig. 8, Plate V). The second *inside* rabbit and another *outside* rabbit then began to change. At this time the third rabbit outdoors was lagging behind those inside. By Nov. 3 the changes were becoming more rapid in all the rabbits except one animal indoors.

The first rabbit which had shown changes on Oct. 23 was by this time nearly white except for a brown patch on the forehead and another behind the ears (Fig. 9, Plate V). This animal was practically as white as he would ever become, (Fig. 10, Plate V), by Nov. 11, when he was skinned.

The last rabbit to exhibit changes was a very young animal, captured late in August. It was kept in the same heated room as the two indoor rabbits. Up to Nov. 11 scarcely any changes were observed except on the

FIG. 22. *Injured White Hair. Entrance of air had caused cells to collapse.*

FIG. 23. *Break-Down of Cells due to Entrance of Air. Bands of melanin in former cell-positions.*

FIG. 24. *Rupture of Cells by Air-Bubbles.*

FIG. 25. *Similar to 24. Some cells, apparently stronger-walled, have escaped destruction.*

FIG. 26. *Damage to Hair at Cut End due to Entrance of Air. Shiny cuticle noticeable.*

FIG. 27. *Brown Weasel Hair. Sides stained rusty red, centre opaque.*

FIG. 28. *White Weasel Hair. Reticular appearance of central core. Cuticle shiny and hyaline.*

FIG. 29. *Central Core Reticular and Breaking Down; Rusty Deposit of Melanin Remaining.*

FIG. 30. *Central Core Vanishing Leaving Hair Hyaline.*

(Photographs by the Author)

ears and feet. This animal did not show definite signs of change until Nov. 11 but six days later it had white rings around the eyes and ears, round patches of white near quarters, a ring around the neck, and the guard hairs whitened over the back. It was then skinned.

All six of the animals were killed at various stages. Two were allowed to turn completely white, two were killed early in the period of change, and the remaining pair at an intermediate stage. It was unfortunate that this had to be done, but the writer felt that he must retain evidence of what had taken place. (Fig. 11, Plate V).

Pigmentation of the Skin in Rabbits and Other Animals

The skin of *L. americanus* is pigmented all the year round on certain parts of the body. The eyelids and lips, and the tips of the ears show pigmentation at all times, but in other parts which are clothed with long hair, the true skin may be devoid of color. Schwalbe, quoted by Dawson (4), says, "at no time of the year were pigment cells found in the corium". This statement is quite incorrect with respect to at least three of the animals which turn white in winter, namely, the rabbit, the ermine and the polar bear. Figs. 12-13, Plate VI, show two pictures taken of a section made on the edge of a rabbits' eyelid. Pigment-bearing cells may be seen in the exposed portion of the eyelid, but, in the other part which was covered with thick winter fur, none are visible. The weasel appears to be in the same class as the rabbit except that his coloring matter is red and not black. In the case of the polar bear, the exposed portions of his skin are a brownish black, but in the Malpighian layer of the hairy portions of the body melanin-secreting cells are present (Fig. 14, Plate VI). It must be remembered that the polar bear keeps his white coat during the summer, hence it is necessary, as a protection from the sun, that his skin should be pigmented.

A pigmentary layer would therefore appear to be required, not in the heavily furred areas, but in the exposed parts of the body. In the rabbit and ermine, the hair roots may partially or completely take the place of the usual pigmentary layer in the skin. At one time, the writer thought that the skin itself as well as the hair roots must be pigmented, but such need not be the case at all; with ermines and rabbits the hair roots, being so numerous, may noticeably darken the skin. Fur dealers watch for this black and patchy coloration which is especially marked in the spring and when it is visible they declare a skin unprime. The statement made by Schwalbe that "the hairs of animals whose coats undergo a seasonal variation; when white, no pigment is found in the hairs of epidemis or corium" implies that these animals become albinotic in winter, that is to say, completely devoid of pigment. This is entirely wrong. The presence of pigment in the hairs may easily be demonstrated and, though the hair roots do cease to produce melanin for a period, the exposed parts of the body are pigmented. Summerhayes and Elton (16) have just brought out an interesting fact concerning the

ivory gull *Pagophila eburnea*. This bird is pure white externally and "is to the polar bear what the jackal is to the African lion". Like the bears, the bird lives on the ice all the year and consequently its white coloration may serve the same purpose as in bears.

The observations which follow will show that environmental factors can be ruled out, because captive rabbits kept indoors entirely away from sunshine and cold, changed color like those which lived outdoors. It seems quite clear then that rabbits become white because they have acquired this habit. The change is probably controlled by internal secretions. This idea gains support from the fact that the hair roots lying below the whitening parts of the hair lose their pigment and become shrunken and frayed out at the ends a few days before the hair itself turns white; and further evidence is found in the fact that the change stops completely when the skin, or the hair by itself, is severed from the body.

Mention should also be made of a certain amount of uneasiness manifested by the rabbits during the period of rapid change, which suggests a bodily reaction.

The autumn change from brown to white follows certain areas with great regularity. The first noticeable changes are on the ears and feet which whiten during the period from the latter part of September to the end of October. Then the changes become more rapid. The next parts to whiten are rings round the eyes, the base of the ears and a patch on the lower part of the nose. Scattered patches of white appear on the flanks, and a larger whitening area just in front of the hind leg in the lumbar region. The guard hairs next turn white and the process advances rapidly over the body. The last places to turn are an area on the forehead, and another which runs along the neck from behind the ears to the top of the shoulders. The period of rapid change, counting from the end of October until it is complete, takes only about twenty days. During the first part of this period, the alterations come day by day with great rapidity. As an example, the record for the last rabbit in the series is given:

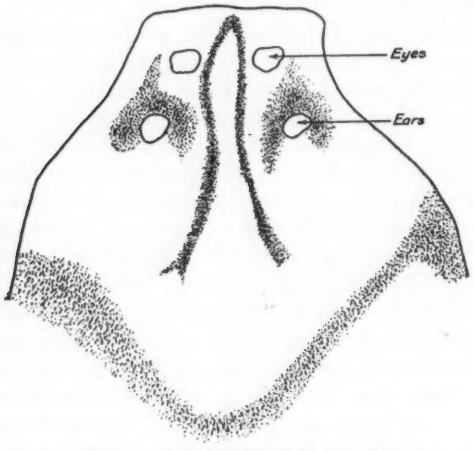


FIG. 15. *Pigmentation of Head and Neck in Changing Rabbit.*

Nov. 16. Unmistakable signs that color is changing. Two small patches of white on lower side of eye, about the size of a grain of wheat. A little whitening around neck. Nose white.

Nov. 17. White extending up flanks. Rabbit uneasy.

FIG. 31. *Disappearance of Central Core.*

FIG. 32. *Under Side of Weasel Skin. Similar grouping of roots to that of pig-skin.*

FIG. 33. *Silver Fox Hair. Blue-grey near skin. Coarsely granular melanin in cuticle.*

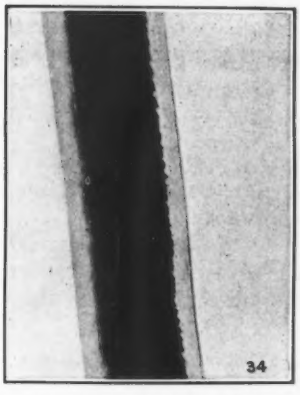


FIG. 34. *White Portion of Same Hair as in 33.*

FIG. 35. *Black Terminal Part of Same Hair as in 33 and 34.*

FIG. 36. *Air Bubbles in Fox Hair*

(Photographs by the Author)

Nov. 22. White ring round neck well defined, also rings round eyes and ears (Fig. 15).
Roundish patches of white near quarters. Guard hairs white. Fur along back still quite dark. Rabbit killed.

Microscopic Changes in Hair Roots.

In the months of April and May, the hair roots are so filled with pigment that they are entirely opaque. They have a large bulbous enlargement and the hair bends up at an acute angle (Fig 16, Plate VI). In October and November, the roots are not so bulbous and the pigment in them is scattered and small in amount. Some of the roots are colorless and shrunken, with frayed ends (Figs. 17-18, Plate VI). In December, the skin is white and there is no color in the hair roots. On Jan. 24, a small percentage of hair roots show color (Fig. 19, Plate VI). In February and March, the production of melanin is marked. In concluding this paragraph on pigmentation, an unusual case of coloration in a pig may be mentioned. The animal was a Yorkshire and on its back were some small slightly blackened spots. On examination of a section it was found that the blackness was caused by pigmented hair roots; no pigment-bearing cells could be discovered in the skin. These pigmented roots in the skin gave precisely the same effect as they do in rabbits.

Physical Changes in the Hairs

At least three possible explanations may be offered for the white appearance of hairs. In Fig. 20, Plate VI, may be seen minute gas bubbles grouped, to a large extent, around the four sides of the cells. These gas bubbles have been noticed in the hairs of both indoor and outdoor rabbits and also in albinos. It is considered that the large amount of total reflection suffered by light incident on the surface of the bubbles may be in part responsible for the white color of these hairs.

Another very noticeable alteration in hairs which have turned white is a shrinking of the core. This causes the cuticle and cortex to become hyaline and the effect is as if the hair were encased in a bubble. The hyaline cuticle and cortex normally cause the white appearance of the polar bear and of the tip of a fox's tail. Possibly the best proofs that the hyaline cuticle and cortex are alone sufficient to account for the white appearance are to be found in the silver fox and the ermine. If the silvered hairs of the fox are examined, it will be seen that they are of three colors. Next to the skin they are blue. In this zone, the cuticle and cortex are speckled with dots of melanin, which disappears where the hair turns white; the tip, which is wholly black, is opaque. Where the hairs of the ermine are white, the cuticular strip is shiny, but where they are brown, rusty spots and streaks are visible throughout it.

The only practical use the writer has found so far for his studies on hair, occurred in connection with the theft of a horse. This animal had on its forehead a white albinotic star, which the thief attempted to obliterate by applying a brown dye. When the hairs were examined under a microscope,

it was quite easy to see the staining material adhering to the outer surface of the shiny transparent cuticle and cortex. In some cases, the dye had not completely covered the surface of the hair; in such places the white hair emerged abruptly from the dyed part.

The third alteration noticed in the hairs is when air or liquids gain entrance to the core, causing the rows of cells to break down. This has been witnessed several times, in one case while a photograph was being taken (Figs. 22 and 24, Plate VII). This form of injury can be readily seen near the free tips of hairs and also near cut or broken ends. The bubbles which gain entrance cause the dry cornified cells to explode rapidly and the bubbles dart about erratically in the centre of the hair. Some of the cells are more resistant than others. Consequently, when the process is complete, unaffected cells may remain, or nothing is left but the cuticle and cortex, inside of which are faint indications of the spaces the cells occupied, and rows of pigment granules which were formerly held in the walls (Figs. 21-26, Plates VI and VII). The collapse of a rabbit hair reminds one of the fading and shrinking of a flower, from an apparently firm and fleshy-looking object to a small fraction of its former size. Desiccation must have some effect on the durability of rabbit hair because so many of the white autumn hairs have a collapsed and irregular appearance; but, though the physical changes are so marked, they are not the primary factor in the alteration. This point is discussed later in the paper.

Comments on Autumn Changes of Color

Allen (1), Nelson (11), Seton (15), and Anthony (2) state that in the autumn the summer hair is shed and is replaced by a white (albinotic) coat. Pennant (12) says: "From Hudson's Bay, as low as New England, these animals, at approach of winter, receive a new coat, which consists of a multitude of long white hairs, twice as long as the summer fur, which still remains beneath. About the middle of April they begin to shed their winter covering." Richardson (14) writes: "After a careful examination, however, of many specimens in different states, I agree with the clerk of the California[1] in thinking that the change to the winter dress takes place by a lengthening and blanching of the summer fur; whilst the change in the beginning of summer consists in the winter coat falling off during the growth of new and colored fur." Merriam (10) agrees that Richardson's opinion "comes very near the truth, but does not express the whole truth. The first clause is absolutely correct; for in the fall the change certainly does occur 'by a lengthening and blanching of the summer fur,' the individual hairs changing color after the first fall of snow. This species, like the great majority of mammals, is clothed with two kinds of hair—a fine soft fur which densely covers all parts of the body, and longer, stiffer hairs, scattered through, and projecting beyond, the former. These long hairs are black in summer and white in winter. In the fall of the year, when the change begins, they become white at the tips

[1] *Voyage in search of a North-West Passage.*

first, the black gradually fading from above downwards until the entire hair is white. In spring the process is reversed, the exposed portion of the long hairs becoming black (though the extreme tip sometimes remains white until the change is far advanced) which color gradually extends downward, at the expense of the white, until the entire hair is black. Sometimes the displacement of the white is temporarily interrupted, the two colors appearing in alternate zones, and, during the latter part of March, when the body of the animal is still white, it is not uncommon to find hundreds of black hairs scattered over the back, many of them with the extreme apices, and a narrow zone between the middle and base, white. In fall or early winter the soft fur becomes tipped with white, the white portion increasing somewhat in length and diameter. In spring a curious phenomenon takes place. The white portion of the fur loses its vitality, becomes brittle, and breaks off on slight friction, so that the animal, in brushing through the undergrowth, soon rids himself of it. As a rule the long hairs change first[1]. Both in spring and fall the time of the change seems to be governed by the presence or absence of snow, and is not affected by the temperature. It occurs independently of the moult, and the new hairs assume the prevailing color of the animal, or the color toward which it is tending at the time of their appearance."

At no time during the year has the writer seen a heavy moult, such as one finds in tame animals, nor any evidence of matting[2] as it occurs in long-haired tame rabbits. Undoubtedly, the greatest amount of hair is lost in the spring, and a certain percentage of the coat is loose and ready to fall out not long before the autumn change of color. Even after the hair has become white it is possible to pull out dead hairs which have all the appearance of belonging to the spring coat; they are black at the tip and have a light yellow zone. It is probable that these are the hairs referred to by Allen (1) as parti-colored hairs. However, though some shedding goes on during the change, it represents only a very small percentage of the total hair covering. It has never seemed probable that the rabbits would shed all their hair in the autumn and replace it during the early part of winter when they are in need of warmth. Furthermore, it seems an impossibility for a pigmented animal like a rabbit to grow white hair unless it is an albino. When the white hair is examined under a microscope, the pigment can still be found in it, effectually disposing of the idea of a new growth of white hair. Another fact which is contrary to the theory of new growth is the comparatively short period during which it occurs. Welch (17) believed that the hair changed color, but he wrote, "Is it dependent on an abstraction of pigment, an alteration, or a new deposition ?", which clearly shows that he did not understand the change.

[1] *Specimens in my museum, killed in Lewis County, December 1st, March 21st and April 3rd, well illustrate the above described conditions of pelage. In spring, while the change is in progress, the attachment of the white tips is so feeble that hundreds may be blown off at a single puff. The change occurs more or less irregularly over the greater part of the body, but is usually symmetrical on the head, giving rise to a very pretty pattern.*

[2] *Dr. R. M. Anderson recently showed the writer the skin of an Arctic hare which had become matted and felted just before shedding time; so it is possible that this may also occur in snowshoe rabbits.*

Probably the most convincing proof that the change takes place in existing hairs is to be found in the skin itself when the hair roots are examined. The fact that the roots cease to function as the hair turns white, and that it is a progressive change, offers conclusive evidence that the alteration is destructive. The reasoning is similar to that advanced by Dwight (5) concerning feathers in birds. Further supporting evidence lies in the variation in the times at which the rabbits changed color. True, the difference between them was only a matter of a few days, in most instances, but there was an extreme difference of thirty days, considering the time of the first rabbit and that of the last, during the period of rapid change. This certainly indicates that outside factors do not control the change, but that it occurs as soon as the rabbits are in the proper condition. It now appears quite certain that color change in rabbits is a matter of long inheritance. It is on a par with Darwin's observation that the thickness of fur in northern animals is inherited and that it persists even when they are removed to a warm climate.

Physical and Chemical Aspects of Hair Color

Some physical and chemical tests to restore the color in hair or to cause it to vanish were sometime ago suggested by Dr. Alty and Dr. Thorvaldson of the University of Saskatchewan. The results of only one or two of these experiments will be mentioned because it has since been proved that environment plays no part in the change of hair color. Nevertheless, knowledge of the physical differences between normally colored and white hair is valuable in spite of the fact that what brought them about is still a matter for speculation.

Pigmentary colors differ from structural colors, the latter being dependent on structure alone. In rabbit hair, owing to the cellular content and pigmentation, the colors may be described as belonging to both classes. Structural causes of white coloration are found in the gas bubbles and numerous reflecting surfaces.

Bubbles may be rapidly formed in the hair by boiling it in water. The color changes quickly, and on being dried, the hair twists and becomes misshapen. To prove that white rabbit hair contains much gas is simple. All that is required is to immerse the hair in a liquid and then to pump the air out of the container. The liquid soon becomes full of bubbles; in fact, it has the appearance of boiling.

One of the methods tried in an endeavor to restore color to whitened hair was to immerse it in pure carbolic acid. Gas was evolved and bubbles might be seen adhering to the hairs. In twenty-four hours the tips, viewed in certain lights, had assumed a brownish appearance. The carbolic acid also caused

the colored bands below the white to darken. The writer is aware that carbolic acid may easily become oxidized; it was here used as a clearing agent to make the hair as transparent as possible.

Perfect recoloration of white hair would appear to be impossible due to the structural changes that have already occurred in it.

Variations in the Coats of Other Animals

Changes in the Ermine or the Weasel.

Weasel hair differs very much from rabbit hair; it is more like horse hair in appearance. In summer, the central core is opaque, and the wide strip of cuticle and cortex surrounding the hair is thickly dotted with rusty brown melanin, some of the particles being spindle-shaped. After the hair turns white, the central core, composed of narrow continuous bands forming a reticulum, becomes less dense. The cuticle and cortex are now clear and hyaline, but under high magnification slight traces of the rusty color may still be found. How the pigment disappears in winter is difficult to understand. Still more difficult is it to determine how the central core may vanish, apparently leaving nothing behind but the shiny shell of a hair (Figs. 27-32, Plates VII and VIII).

As far as the writer has been able to ascertain, weasels become white a little in advance of rabbits. He has seen them change color as early as Oct. 25.

The Silver Fox

Through the kindness of Dr. A. Kingscote, the writer has received a fine collection of fox fur, taken at regular monthly intervals for a whole year. The study of this material has not yet been completed but a description of the silvered hair will be given here as it offers such excellent proof that only small physical changes are necessary to make different colors. Figs. 33-36, Plate VIII, are photographs taken of a single hair. The color near the skin was a grey blue. In this portion, the wide cuticle and cortex surrounding the hair were thickly dotted with black melanin. A little further up the hair, the cuticle and cortex cleared abruptly and became very shiny. This was the white portion of the hair. Nearer the tip, which was coal black, the hyaline part narrowed down abruptly into a perfectly opaque region. A comparison was made between the white band and pure white hair taken from the tail of the same animal. There appeared to be no difference whatsoever between the two.

The White-Tailed Jack Rabbit.

The white-tailed jack rabbit (*L. campestris*) has not yet been thoroughly studied. It may be said, however, that there is a general similarity between it and *L. americanus*, though it lives much more in the open. These rabbits change color in the same way as *L. americanus* but more completely. Late

in November the hair is tricolored in some parts of the body, especially the neck, the colors being blue, reddish-brown and white. Along the back only a slight tinge of brown is visible. It has been noticed that the guard hairs turn white before the rest of the coat, and that shedding of the spring hairs continues up to the time the coat becomes white, and even afterwards. The amount of hair falling out as the coat changes color is only a small percentage of the total hair cover.

Arctic White Foxes.

Portions of unprime white fox skins taken early in the winter show color changes very similar to rabbits. Degenerating hair roots are found almost indistinguishable from those of a rabbit. It is evident that the alteration of color in the fur is preceded by loss of function on the part of the hair roots.

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